Observations of Scope For Growth, Aspartate Aminotransferase activity and Glucose-6-Phosphate Dehydrogenase activity in *Mya truncata* and *Serripes groenlandicus* exposed to various concentrations of chemically dispersed crude oil in the DIAND/BIOS 1983 tank study.

by

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1.0 INTRODUCTION

Observations made during the 1981 field season of the BIOS program showed that both Mya truncata and Serripes groenlandicus accumulated spill-derived petroleum hydrocarbons to high levels (300-500 ppm). It was apparent that Serripes groenlandicus accumulated higher levels of petroleum hydrocarbons than Mya truncata. At the same time it was apparent that Serripes groenlandicus was more profoundly affected by the accumulated petroleum as evidenced by their leaving their burrows even when exposed to relatively low concentrations of chemically dispersed petroleum.

Controlled observations made during the 1982 field season showed that the behavioral reactions observed during the 1981 field season were reproducible in the laboratory.

Two major questions emerge from this data: (1) Are the observed behavorial differences between the two species related to differences in their respective filtration rates; (2) To what extent are the accumulated hydrocarbons affecting the physiological well–being of the two species. The following report gives the results of a research program which was designed to address both of these questions.

2.0 MATERIALS AND METHODS

Study Plan: The basic study plan was to determine the following parameters on populations of Mya truncata and Serripes groenlandicus that had been exposed to a variety of conditions. The first group of parameters to be measured was the elements of scope-for-growth, i.e. filtration rate, respiration rate as well as assimilation ratio. In addition, activity of Aspartate Amino-transferase (AAT) , an enzyme important in the metabolism of protein, and Glucose-6-phosphate dehydrogenase (G-6-P) , an enzyme important in the metabolism of carbohydrate, were determined. Experimental animals were collected from wild populations of each species. In addition, animals held in flowing seawater in the laboratory were exposed to a number of treatments:

1. No treatment (control)
2. Exposure to a dispersed oil regime similar to that observed in Bay 9 (10 ppm - 6h, 100 ppm - 6h, 5 ppm - 6h: Bay 9 Sequential Exposure).
3. Exposure to a dispersed oil regime similar to that observed in Bay 10 (0.5 ppm - 6h, 5 ppm - 6h, .02 ppm - 6h: Bay 10 Sequential Exposure) .
4. Constant exposure to 50 ppb dispersed oil (long term 50 ppb).
5. Constant exposure to 500 ppb dispersed oil (long term 500 ppb).

2.1 Sampling Strategy: Samples of experimental animals were collected four times during each experiment for each species:

O hours, 18 h, 7 days and 14 d. Samples were not taken of the control animals at 18 h. Samples were analyzed for body burden of petroleum (Boehm, 1984) , enzyme activity, and scope-for-growth.

2.2 Crude Enzyme Preparation:

Because of limitations on the capacity of the exposure systems, very limited numbers of animals were available for enzyme analysis. Animals to be used for enzyme analysis were frozen in the field and transported to the laboratory in a frozen state. Samples were thawed immediately prior to analysis. Tissue from two or more, when available, individuals was used for each assay. The tissue was combined with three parts w/v of an ice cold buffer solution consisting of .01 M Trienthanolamine-HCl (Tris-HCl) and 2mM Ethylenediaminetetraacetic acid (EDTA) and then ground thoroughly in a Virtis homogenizer. This preparation was then centrifuged at 11,000 g at 4 'c. The supernatant was used as the crude enzyme extract. The extracts were analyzed immediately. Protein content of the extract was determined by the Biuret test using bovine serum albumen as a standard.

2.3 Glucose-6-Phosphate dehydrogenase activity:

The method used for the assay of glucose-6-phosphate activity is adapted from that of Gould 1977. Assays were performed at 340 nm using a double beam spectrophotometer (Perkin Elmer Lambda 3) with the chamber temperature at 25 'C, using 1 cm cuvettes. Absorbance was recorded on a strip chart recorder. Reaction rates were read beginning 90 - 120 seconds after the start of the reaction. The reaction rate was calculated using the steepest linear

portion of the curve.

For the G-6-P assay each cuvette contained 2.45 ml tris(hydroxymethyl) aminomethane buffer, .1 M, pH 8; .1 ml nicotinamide adenine dinucleotide phosphate, 8 mg/ml; .1 ml d-glucose-6-phosphate disodium salt hydrate, 15 mM; 0.15 ml $MgCl_2 . 6H_2O$, 50 mM; .1 ml maleimide, 8 mg/ml H_2O. The reaction was started by addition of .1 ml of the crude enzyme extract. The unit of activity was .001 change in absorbance/min/mg protein. Each sample was run against a cuvette containing all reagents except the crude enzyme preparation. Each assay was performed in triplicate.

2.4 Aspartate Aminotransferase activity:

The assay procedure is adapted from Thurberg et al. 1977. These assays were performed using the same instruments and cuvettes as for G-6-P assays. Reaction rates were determined by measuring decreased absorbance over 7 minutes; the steepest linear portion of the curve was used to calculate the reaction rate. The unit of activity was .001 change in absorbance / minute / mg protein.

The reaction mixure contained 2.65 ml phosphate (KH_2PO_4) buffer, 0.1 M PH 7.5, which also contained 0.25 M L-aspartic acid (neutralized with KOH prior to addition) ; .1 ml reduced nicotinamide adenine dinucleotide, 9 mg/ml .1 M phosphate buffer; .05 ml crude enzyme extract. The reaction was started by addition of . 2 ml alpha-ketoglutarate, .15 M, neutralized with KOH. Each sample was run against a blank containing all reagents except

alpha-ketoglutarate. Each assay was performed in triplicate.

2.5 Scope for Growth Methods:

Methods used to determine scope for growth were adapted from Gilfillan et al., 1976. After acclimation, respiration rates were determined for four animals from each population using a Gilson Differential Respirometer. Respiration rates were determined at constant temperature in a flowing seawater bath which was always within 2-3 'C of the temperature of the ambient water. After the respiration measurements the animals were placed in individual, labeled, paper envelopes and frozen. Subsequently, the animals were freeze dried and the dry tissue weighed (+/- 0.1 mg) . As a result of limitations in the number of experimental animals not all animals could be frozen and weighed. The lengths of all experimental animals were recorded; linear regression of length against weight allowed estimation of the missing values. Respiration rates were calculated as ul 02 consumed/mg/hr.

In order to determine filtration rates one animal was placed in each of 5x4 liter beakers filled with glass fiber filtered seawater to which sufficient Dunalliella tertiolecta had been added to equal the food concentration observed in the ambient water. Prior to placing the experimental animals into the beakers 5x25ml samples were drawn and the number of food particles counted using a Coulter model ZBI particle counter. After six hours (Mya truncata) or four hours (Serripes groenlandicus) the animals were removed and five additional 25 ml samples were drawn

and the number of food particles determined as above. Feces produced by the animals were collected on tared, pre-combusted glass fiber filters. All experiments were determined at running sea water temperature. Animals were freeze dried and weighed (+/- 0.1mg). As a result of limitations in the number of experimental animals not all animals could be frozen and weighed. The lengths of all experimental animals were recorded; linear regression of length against weight allowed estimation of the missing values. The volume swept clear of particles was calculated from the relation:

$$V\text{-}v(\ln C_o - C_t) / t$$

Where V = volume swept clear of particles in liter/hr; v = volume of the experimental container; Co = initial food concentration; C_t = final food concentration; t = duration of the experiment in hours.

The filter with the feces was washed with distilled water to remove salt, freeze dried and weighed (+/- 0.01mg) to give a dry weight. The filter was then ashed for 10 minutes at 600 'c to give an ash weight. The organic fraction of the feces was determined as ash-free dry weight divided by total dry weight. A filter containing food was treated the same way to yield organic fraction of the food. Assimilation efficiency of the animals was determined from the following relationship:

$$\text{Assimilation efficiency} = (A - B)\backslash (1-B) * A * 100$$

A = organic fraction of food
B = organic fraction of feces

Values of respiration and filtration rate observed will vary depending on the size of the experimental animals. Before comparisons between stations can be made it is necessary to reduce the data to some common measure. One way in which to overcome unavoidable differences in size between groups of experimental animals is to express results in terms of a standard animal.

The work of Zeuthen 1947; 1953 and Hemmingson 1950, 1960 showed that many of the rate functions of nearly all marine poikilotherms vary logarithmically with body weight. As a result, relations of this type are frequently used to calculate values in terms of standard animals (Ansell and Lander, 1967; Gilfillan et al., 1977) . The relationship between the common logarithm of the animals' dry weight and either the volume of oxygen consumed or the volume of water filtered was used to calculate either the volume of oxygen consumed or the volume of water filtered by a standard animal (Serripes groenlandicus, 1000 mg; Mya truncata, 1400 mg) . Values chosen for the standard animals were the mean of the weights of all animals used.

Once standard values for respiration and filtration are obtained for standard animals, it is possible to convert them to carbon units and calculate scope for growth for the animals in question. A value of .85 has been assumed for the animals' respiratory quotient which is typical for an animal metabolizing a mixture of fat and carbohydrate. In view of the fact that both

bivalves are herbivores it is possible that the R.Q. will be higher than 0.85. However, an assumed value of 0.85 will not introduce error because this is a comparative study. Assuming an R.Q. of 0.85, carbon respired in ug/hr is calculated as 0.43 x ul 02 consumed/hr.

The amount of carbon consumed by the animals(gross carbon) was calculated as the filtration rate of the standard animal in l/hr x 500 ug C /liter.[1] Using a constant amount of available food facilitates comparison of data between sampling dates and introduces no error in a comparative study.

Net carbon was calculated as gross carbon x assimilation ratio. Scope for growth was calculated as net carbon - respired carbon.

[1] Mean concentration of carbon in food samples.

3.0 RESULTS

Table 1 shows data for the elements of scope for growth (SFG), assimilation (ASSIM), filtration rate (FILT), respiration rate (RESP) as well as for the activity of AAT and G-6-P for all groups of Serripes groenlandicus which received no oil treatment. The mean of all these values of the individual parameters was used as an estimate of the time O, or control rate in the tables and figures which follow. This method was used to get the best possible estimate of unperturbed physiological rates in these animals.

Table 2A shows values of the elements of scope for growth as well as enzyme activity values for Serripes groenlandicus which were exposed to the Bay 9 sequential exposure to dispersed oil. Also shown are data for the body burden of aromatic hydrocarbons (Boehm, 1984). The scope for growth data are shown graphically in Figure 1A; the enzyme data are shown graphically in Figure 1B. As can be seen from Figure 1A, the major effect on SFG is brought about by a reduction in filtration rate. Values for SFG do not recover to control levels within 14d. 'The activity of both AAT and G-6-P are greatly increased following oil exposure (Fig. 1B); activity values return to control levels within 14 d.

Table 2B shows values for the elements of scope for growth as well as values for enzyme activity for Serripes groenlandicus exposed to the Bay 10 sequential exposure. Also shown are values

for the body burden of aromatic hydrocarbons (Boehm, 1984) . Data for SFG are shown in Figure 2A. As can be seen there is a significant, but smaller, reduction in SFG following oil exposure. This reduction in SFG is brought about by reduced filtration combined with increased respiratory loss. Observed values for SFG return to "control" levels within 14d. Data for AAT and G-6-P activity are shown in Figure 2B. For both enzymes there is a reduction in activity following exposure to dispersed oil; activity returns to "control" levels within 14d.

Figure 3A shows SFG data for Serripes groenlandicus which were never exposed to oil. Relatively little change over time is visible. Figure 3B shows enzyme data for the same groups of animals. No significant change occurs between 0 and 7d; following that, the data get noisier. In the sequential exposure experiments most of the physiological changes occurred within the first 7d.

Table 3A shows values for the elements of SFG as well as values for enzyme activity for Serripes groenlandicus exposed to 500 ppb dispersed oil for 14d. Values for the body burden (gut) of aromatic hydrocarbons are also shown (Boehm, 1984) . Data for SFG are shown in Figure 4A: SFG is reduced throughout the exposure period. The reduction in SFG is largely brought about by reduced filtration. Data for enzyme activity are shown in Figure 4B. These data are very noisy but there is a trend toward increased enzyme activity with increased time of exposure.

Table 3B shows values for the elements of SFG as well as values for enzyme activity for Serripes groenlandicus exposed to 50 ppb dispersed oil for 14d. Values for the body burden (gut) of aromatic hydrocarbons are also shown (Boehm, 1984). The SFG data are shown graphically in Figure 5A. Data are only shown for the first 7 days because extensive mortality in the experimental population precluded further measurements. Little effect on SFG is observed. Figure 5B shows enzyme activity data for the same group of animals. These data are relatively noisy; there is a trend for higher values of enzyme activity with increased time of exposure.

Figure 6A shows SFG data for Serripes groenlandicus never exposed to dispersed oil. Little effect on SFG is observed. There are no data for SFG or assimilation for day 14 because insufficient fecal matter was available to determine assimilation rates . Figure 6B shows enzyme activity for the same group of animals. Little change is seen until after day 7. This pattern is similar to that seen in the sequential control series.

Table 4 shows data for the elements of NETC as well as for enzyme activity for Mya truncata which received no oil treatment. NETC which equals carbon consumed x assimilation ratio was used in panel A because of unreliable respiration measurements obtained using these animals. The mean of all these estimates of the individual parameters was used as an estimate of the time O, or "control" rate in the tables and figures which follow. This was done to get the best possible estimate of unperturbed physiological rates in these animals.

Table 5A shows elements of NETC as well as enzyme activity for Mya truncata subjected to the Bay 9 sequential exposure to dispersed oil. Also shown are values for the body burden of aromatic hydrocarbons (Boehm, 1984) . Elements of NETC are shown in Figure 7A; NETC decreases in the immediate post exposure period and then returns to "control" levels within 14d. The changes in NETC are brought about by decreased filtration in the immediate post exposure period. Figure 7B shows enzyme activity for the same group of animals. No real trend is visible in the data for AAT or G-6-P.

Figure 8 shows enzyme data for Mya truncata subjected to the Bay 10 sequential exposure to dispersed oil. The activity of both enzymes decreases in the post exposure period and recovers to "control" levels after 14d.

Figure 9A shows NETC data for Mya truncata which were never exposed to dispersed oil. Relatively little change is seen until day 14. Figure 9B shows enzyme data for the same group of animals; there is a trend toward lower activity as time passes. This is particularly true for G-6-P.

Table 5B shows NETC data as well as enzyme activity data for Mya truncata exposed to 500 ppb dispersed oil for 14 days. Body burden (gut) of aromatic hydrocarbons is also shown (Boehm, 1984) . These data are shown graphically in Figures 10A and 10B. No clear trends are evident.

Table 6 shows data for enzyme activity in Mya truncata subjected to the Bay 10 sequential oil exposure as well as for Mya truncata exposed to 50 ppb dispersed oil for 14d. Data for the body burden of aromatic hydrocarbons are also shown (Boehm, 1984). Figure 11 shows enzyme data for Mya truncata exposed to 50 ppb dispersed oil. These data are noisy; the trend is for higher enzyme activity with increased time of exposure.

Figure 12A shows elements of NETC for Mya truncata held in the same way as the animals exposed to 50 or 500 ppb dispersed oil, but which were not exposed. No clear trends are visible in these data. Figure 12B shows enzyme activity data for the same animals. The activity of AAT appears to increase over time; the activity of G-6-P shows no clear trends.

Table 7 shows the results of exponential regression analysis where SFG, AAT and G-6-P in Serripes groenlandicus are taken individually as the dependent variables and the body burden of aromatic hydrocarbons is taken as the independent variable. This method of analysis was chosen because dose-response relations are very often exponential and because the aromatic fraction of the hydrocarbon body burden is that fraction with the greatest biological activity. The analytical results shown in Table 7 show clearly that a high degree of interdependence exists between SFG, AAT, G-6-P (Bay 10 only) and body burden of aromatic hydrocarbons when the data from the sequential exposures are analyzed. No such

relationships occur in the analysis of data from long-term exposures.

Table 8 shows results of exponential regression in which SFG, AAT, and G-6-P in Mya truncata are taken as the dependent variables and the body burden of aromatic hydrocarbons is taken as the independent variable. A high degree of interdependence exists between NETC and the body burden of aromatic hydrocarbons in the data for the Bay 9 sequential exposure. No Bay 10 sequential exposure animals were run. No other significant interdependence was found.

Table 9 shows the relation between filtration rate in Mya truncata and in Serripes groenlandicus and the body burden of petroleum hydrocarbons accumulated by each species after 14 days of exposure to either 50 ppb or 500 ppb dispersed oil. It is clear from these data that Serripes groenlandicus filters ca 5 times as much water per unit time than does Mya truncata. The final concentration of hydrocarbons attained by Serripes groenlandicus is 8.1 (500 ppb) to 6.7 (50 ppb) times higher than that attained by Mya truncata. It should also be noted that the final concentration of hydrocarbons attained at 500 ppb is 7.7 (M. truncata) to 9.5 (Serripes groenlandicus) times higher than those attained at 50 ppb.

Table 9 also shows the rate of deputation of hydrocarbons accumulated by Serripes groenlandicus and Mya truncata during the sequential exposures in terms of the half-life of hydro-

carbons. It should be noted that the t 1/2 values for both species are very similar in the Bay 9 exposed animals; they are less so for the Bay 10 exposed animals.

TABLE 1. Values for elements of scope for growth as well as values for activity of AAT and G-6-P in un-exposed Serripes groenlandicus.

SAMPLE	ASSIM	FILT	RESP	SFG	AAT	G6P
Wild Aug. 3	2 (4)	.673 (.375)	129 (23)	-48	42.4 (8.4)	6.79 (.77)
Wild Aug. 15	84 (4)	.610 (.475)	121 (50)	203	48.9 (4.5)	8.39 (.21)
Wild Aug. 15	42 (26)	.896 (.640)	121 (88)	137	76.9 (14.7)	10.7 (.9)
Sequential T= 0	98 (2)	2.724 (2.292)	132 (67)	1272	16.8 (0.0)	6.88 (.38)
50/500 T=0	79 (4)	.513 (.519)	133 (52)	146	59.8 (4.6)	14.1 (.1)
Seq. Day 7 Control					52.6 (8.1)	14.7 (.4)
Seq. Day 9 Control	89 (10)	.772 (.422)	158 (67)	275	18.9 (5.6)	2.21 (.76)
Seq. Day 13 Control	89 (5)	.899 (.154)	167 (20)	329	26.7 (7.6)	4.69 (.20)
50/500 Day 8 Control	84 (9)	1.424 (.496)	95 (40)	558	60.6 (3.9)	4.82 (.21)
50/500 Day 13 Control	nd	.917 (.379)	117 (38)	nd	127 (2)	12.3 (.4)

nd = no data

( ) = standard deviation

TABLE 2. Values of the elements of scope for growth as well as values for activity of AAT and G-6-P in Serripes Groenlandicus subjected to sequential exposure to chemically dispersed oil.

A - 13ay 9 Simulation

TIME	ASSIM	FILT	RESP	CFLUX	AAT	G6P	AROMATIC HYDROCARBONS
0	71 (32)	1.05 (.68)	130 (22)	359 (408)	53.1 (34.4)	7.88 (3.88)	0.5 *
18 hours	nd	0.000 (0.0)	115 (38)	-50	154 (16)	18.5 (.2)	12.1
Day 7	70 (3)	.501 (.153)	169 (71)	104	83.5 (6.5)	14.7 (1.1)	6.1
Day 14	75 (21)	.264 (.367)	142 (44)	37	62.8 (7.5)	6.46 (.43)	3.6**

B - Bay 10 Simulation

0	71 (32)	1.05 (.68)	130 (22)	359 (408)	53.1 (34.4)	7.88 (3.88)	0.5
18 hours	100 (0)	.504 (.647)	176 (64)	176	23.3 (10.5)	2.68 (.39)	15.6
Day 7	63 (18)	.939 (.490)	83 (49)	263	46.7 (2.2)	7.66 (.36)	4.1
Day 14	78 (6)	.996 (.590)	41 (30)	370	60.1 (15.9)	11.0 (.5)	3.9**

* ppm wet weight

** interpolated from 7d and 21d data

TABLE 3. Values of the elements of scope for growth as well as values for activity of AAT and G-6-P in Serripes_groenlandicus_ exposed to 50 and 500 ppb dispersed oil

A 500 ppb exposure

TIME	ASSIM	FILT	RESP	CFLUX	AAT	G6P	AROMATIC HDROCARBONS
0	71 (32)	1.05 (.68)	130 (22)	359 (408)	53.1 (34.4)	7.88 (3.88)	0.5 *
18 hours	47 **	.363 (.229)	156 (81)	18	72.6 (8.3)	11.4 (.9)	14.0
Day 7	79 (13)	.389 (.276)	116 (21)	104	22.7 (7.3)	4.33 (.16)	39.2
Day 14	100 +	.261 (.063)	61 (37)	104	97.8 (6.1)	22.4 (.8)	140.0
B 50 ppb exposure							
0	71 (32)	1.05 (.68)	130 (22)	359 (408)	53.1 (34.4)	7.88 (3.88)	0.0
18 hours	52 (26)	1.274 (.422)	123 (35)	278	65.5 (9.6)	14.0 (1.8)	1.5
Day 7	54 +	1.132 (.444)	93 (9)	271	42.8 (10.7)	7.46 (.86)	19.0
Day 14	experiments not run due to narcosis				116 (6)	25.0 (1.0)	18.0

* ppm wet weight
** only one experimental value
+ = composite

TABLE 4. Values for elements of scope for growth as well as values for activity of AAT and G-6-P in un-exposed *Mya truncata*.

SAMPLE	ASSIM	FILT	RESP*	NETC	AAT	G6P
Wild Aug. 3	38 (31)	.204 (.100)	89 (11)	39	48.6 (2.5)	15.2 (2.6)
Wild Aug. 15	92 (9)	.157 (.143)	58	72	54.7 (4.3)	.34 (.35)
Wild Aug. 15					63.8 (4.7)	.17 (.30)
Sequential T=0	97 (1)	.238 (.109)	98	116	24.0 (2.7)	.98 (.17)
50/500 T=0	88 (6)	.116 (.114)	123 (78)+	51	29.3 (2.8)	.97 (.10)
Seq. Day 7 Control					33.0 (1.7)	0.0 (0.0)
Seq. Day 9 Control	84 (6)	.118 (.142)	0 (0)	49	31.4 (.5)	0.0 (0.0)
Seq. Day 13 Control	86 (2)	.331 (.279)	90 (17)+	143	27.3 (.3)	0.0 (0.0)
50/500 Day 8 Control	88 **	.398 (.280)	47	175	52.6 (1.6)	.69 (.30)
50/500 Day 13 Control	84 (11)	.071 (.069)	0 (0)	30	57.7 (2.0)	.40 (.35)

* In many cases, 2 or 3 of the 4 respiration replicates = O. This table reports only non-zero values unless all 4 replicates = O. When 3 of the 4 values = O, no standard deviation is reported.

\+ 2 of 4 values = O (not used in average)

** = composite-feces combined to yield one estimate of assimilation.

'TABLE 5. Elements of scope for growth as well as activity of AAT and G-6-P in Mya truncata exposed to Bay 9 sequential simulation and to 500 ppb dispersed oil.

A Sequential Bay 9 simulation

TIME	ASS IM	FILT	RESP+	NETC	AAT	G6P	AROMATIC HYDROCARBONS
0	82 (18)	.204 (.113)	63 (45)	84 (54)	42.2 (14.7)	.39 (.40)	1.5 *
18 hours	100 (0)	0.000 (0.0)	87	0	49.1 (1.3)	.80 (.16)	50.0
Day 7	80 (2)	.102 (.084)	118	41	50.5 (9.8)	0.0 (0.0)	6.2
Day 14	86 (2)	.156 (.172)	0 (0)	67	33.5 (3.9)	.09 (.16)	6.4**
B 500 ppb exposure							
0	82 (18)	.204 (.113)	63 (45)	84 (54)	42.2 (14.7)	.39 (.40)	1.1
18 hours	93 +	.059 (.049)	69	28	28.8 (2.3)	0.0 (0.0)	5.5
Day 7	nd	.141 (.116)	120 (80)	nd	67.0 (4.0)	.30 (.52)	14.6
Day 14	88 (2)	.074 (.024)	79.7	33	30.8 (.7)	.12 (.10)	24.5

* ppm wet weight
** Interpolated from 7d and 14 d data.
\+ In many cases, 2 or 3 of the 4 respiration replicates = O.
This table reports only non-zero values unless all 4 replicates = O.
When 3 of the 4 values = O, no standard deviation is reported.

TABLE 6. Activity of AAT and G-6-P in *Mya truncata* exposed to the Bay 10 simulation and exposed to 50 ppb dispersed oil.

SAMPLE	TIME	AAT	G6P	AROMATIC HYDROCARBONS
Sequential Bay 10	0	42.2 (14.7)	.39 (.40)	1.1 *
	18 hours	26.6 (1.7)	0.0 (0.0)	15.6
	Day 7	23.0 (.9)	0.0 (0.0)	4.1
	Day 14	40.8 (3.3)	.50 (.43)	4.0
50 ppb	0	42.2 (14.7)	.39 (.40)	1.1
	18 hours	49.5 (2.0)	.24 (.41)	1.9
	Day 7	28.8 (1.6)	.63 (.16)	3.1
	Day 14	77.1 (6.8)	1.07 (.18)	3.4

* ppm wet weight
** interpolated from 7d and 14d data.

TABLE 7. Results of exponential regression[1] of various physiological parameters on the body burden of aromatic hydrocarbons: Serripes groenlandicus.

	A	B	R^2
SEQUENTIAL EXPOSURE			
Bay 9			
SFG	469.8	-1.96	.81*
AAT	47.8	.094	.99**
G-6-P	6.69	.087	.73
Bay 10			
SFG	374.4	-.048	.84*
AAT	62.19	-.061	.88*
G-6-P	10.79	-.084	.82*
LONG TERM EXPOSURE			
500 ppb			
SFG	91.31	-.000027	.0000019
AAT	43.70	.0043	.19
G-6-P	6.76	.0074	.46
50 ppb			
SFG	319.69	-.0092	.39
AAT	59.17	.0089	.046
G-6-P	10.45	.014	.066

* P .05

** P .01

1. Rate = A * $e^{(B * \text{cone. aromatics in body})}$

TABLE 8. Results of exponential regression[1] of various physiological parameters on the body burden of aromatic hydrocarbons: Mya truncata.

	A	B	R^2
SEQUENTIAL EXPOSURE			
Bay 9			
NETC	473.4	-.443	,99**
AAT	40.84	3.60	.19
G-6-P	.0011	.12	.13
Bay 10			
AAT	37.21	-.024	.26
G–6–P	.056	-.901	.43
LONG TERM EXPOSURE			
500 ppb			
NETC	3.66	-.22	.052
AAT	40.34	-1.9	.00014
G-6-P	1.30	.19	.096
50 ppb			
AAT	39.07	.073	.038
G-6-P	.16	.48	.65

** P .01

1. Rate = A * e (B * cone. aromatics in body)

TABLE 9. Relationship between filtration rate, hydrocarbon uptake and hydrocarbon deputation in *Mya truncata* and *Serripes groenlandicus*.

HYDROCARBON UPTAKE	FILTRATION	500 ppb'	50 ppb[2]
Mya truncata	.204	49.2[3]	6.4
Serripes groenlandicus	1.05	400.0	42.0

HYDROCARBON DEPUTATION (half-life)	BAY 9	BAY 10
Mya truncata	6.86 days	10.76 days
Serripes groenlandicus	8.41 days	21.0 days

1. Mean filtration rate in l/h for "standard" animals not exposed to oil.
2. Final gut concentration of hydrocarbons.
3. ppm wet weight.

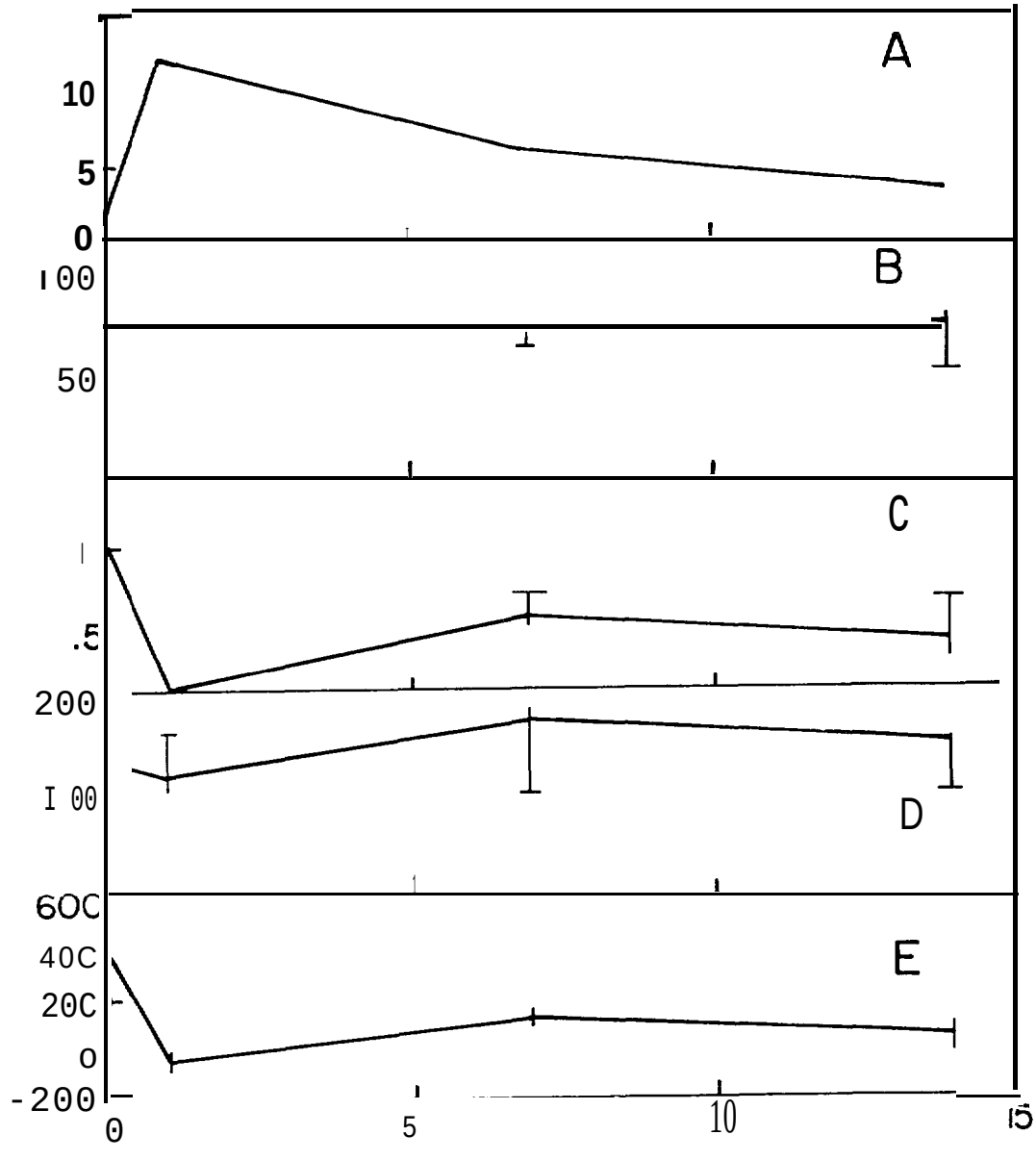

Figure 1A. Concentration of F2 (aromatic) H-C, ppm wet wt. (A); Values for assimilation ratio (B); Filtration rate, l/h (C); Respiration rate, ul/h (D); and scope for growth (E) for Serripes groenlandicus exposed to the Bay 9 simulation. Vertical lines are 1 standard deviation.

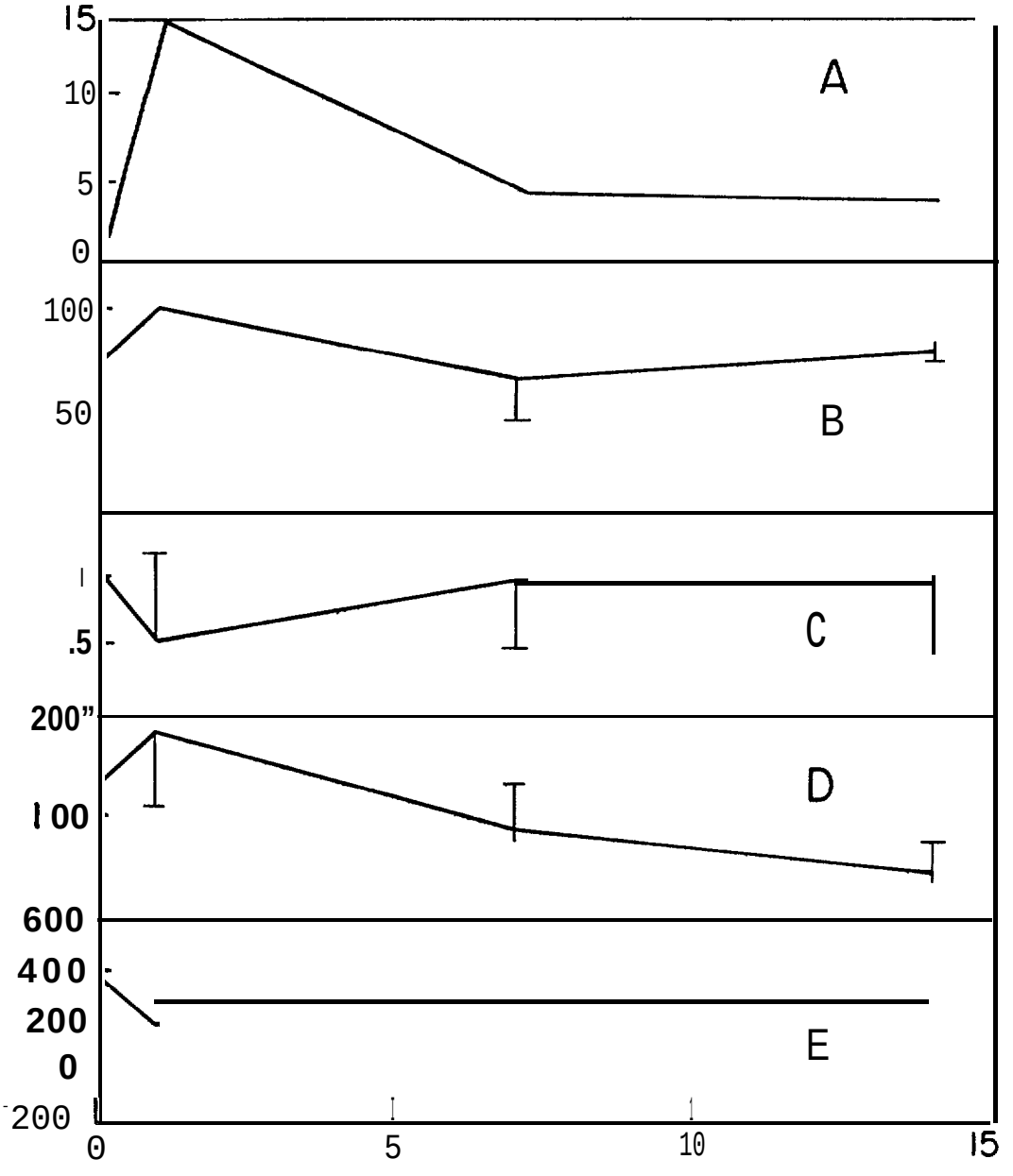

Figure 2A. Concentration of F2 (aromatic) H-C, ppm wet wt. (A); Values for assimilation ratio (B); Filtration rate, l/h (C); Respiration rate, ul/h (D); and scope for growth (E) for Serripes groenlandicus exposed to the Bay 10 simulation. Vertical lines are 1 standard deviation.

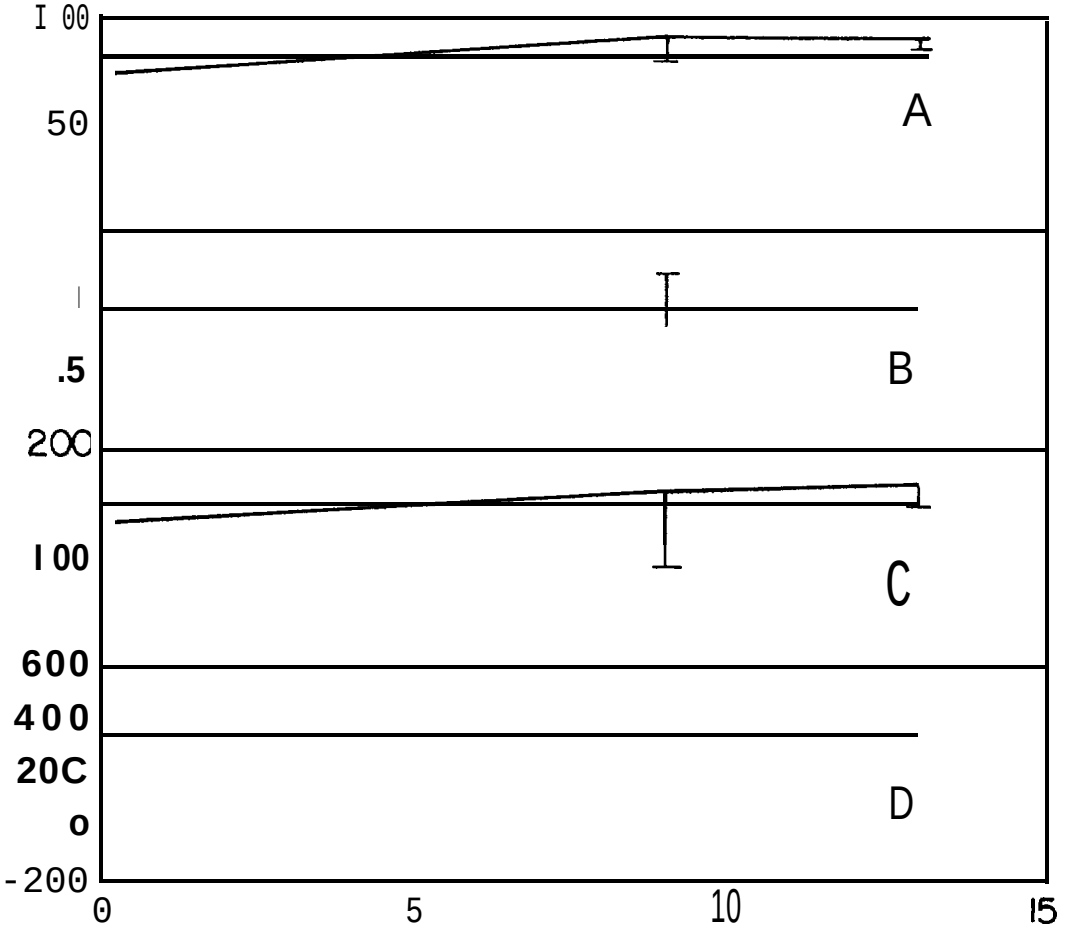

Figure 3A. Values for assimilation ratio (A); Filtration rate, l/h (B); Respiration rate, ul/h (C); and scope for growth (E) for Serripes groenlandicus never exposed to petroleum. Vertical lines are 1 standard deviation.

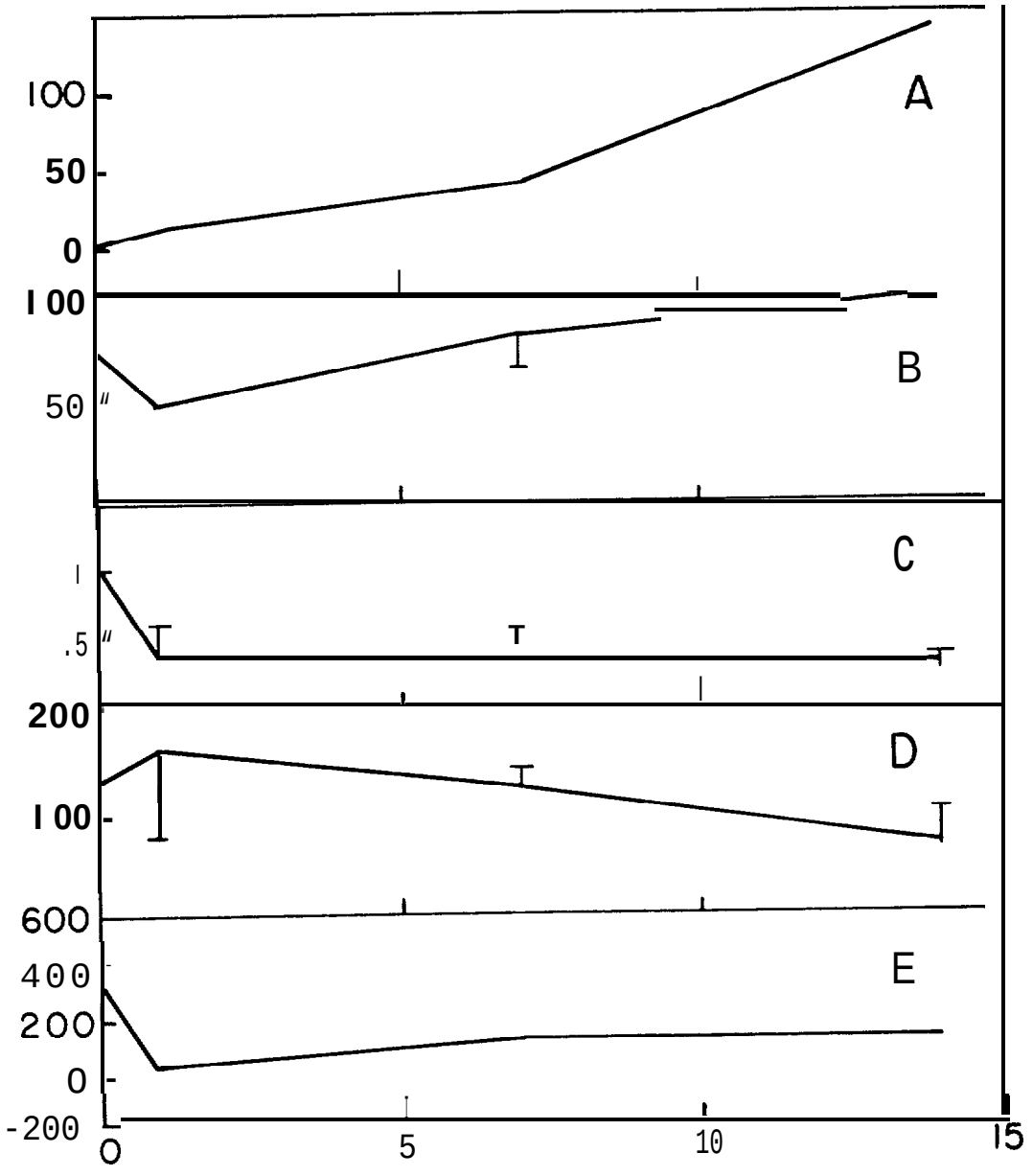

Figure 4A. Concentration of F2 (aromatic) H-C, ppm wet wt. (A); Values for assimilation ratio (B) ; Filtration rate, l/h (C); Respiration rate, ul/h (D) ; and scope for growth (E) for Serripes groenlandicus exposed to 500 ppb chemically dispersed oil. Vertical lines are 1 standard deviation.

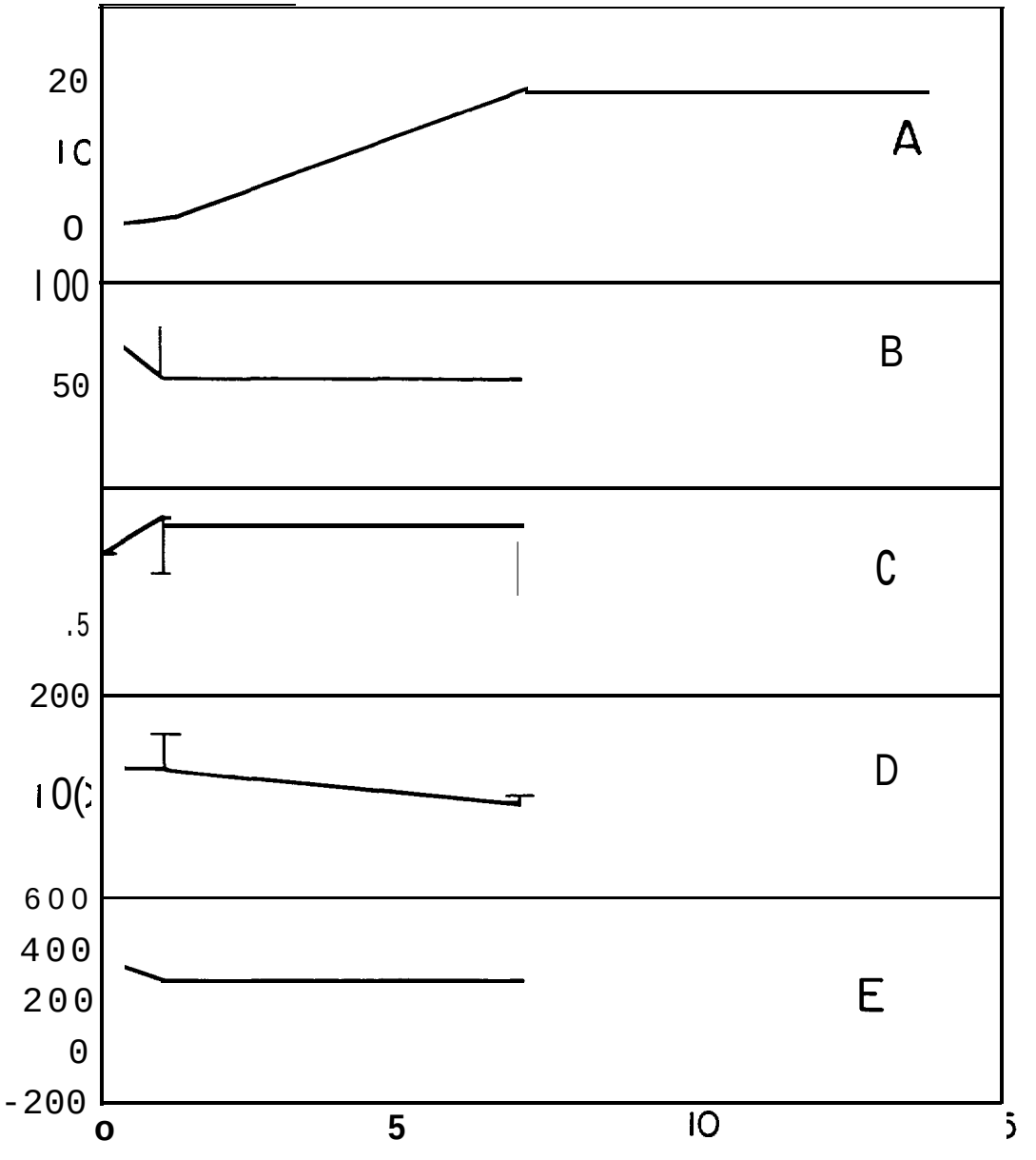

Figure 5A. Concentration of F2 (aromatic) H-C, ppm wet wt. (A); Values for assimilation ratio (B); Filtration rate, l/h (C); Respiration rate, ul/h (D) ; and scope for growth (E) for Serripes groenlandicus exposed to 50 ppb chemically dispersed oil. Vertical. lines are 1 standard deviation.

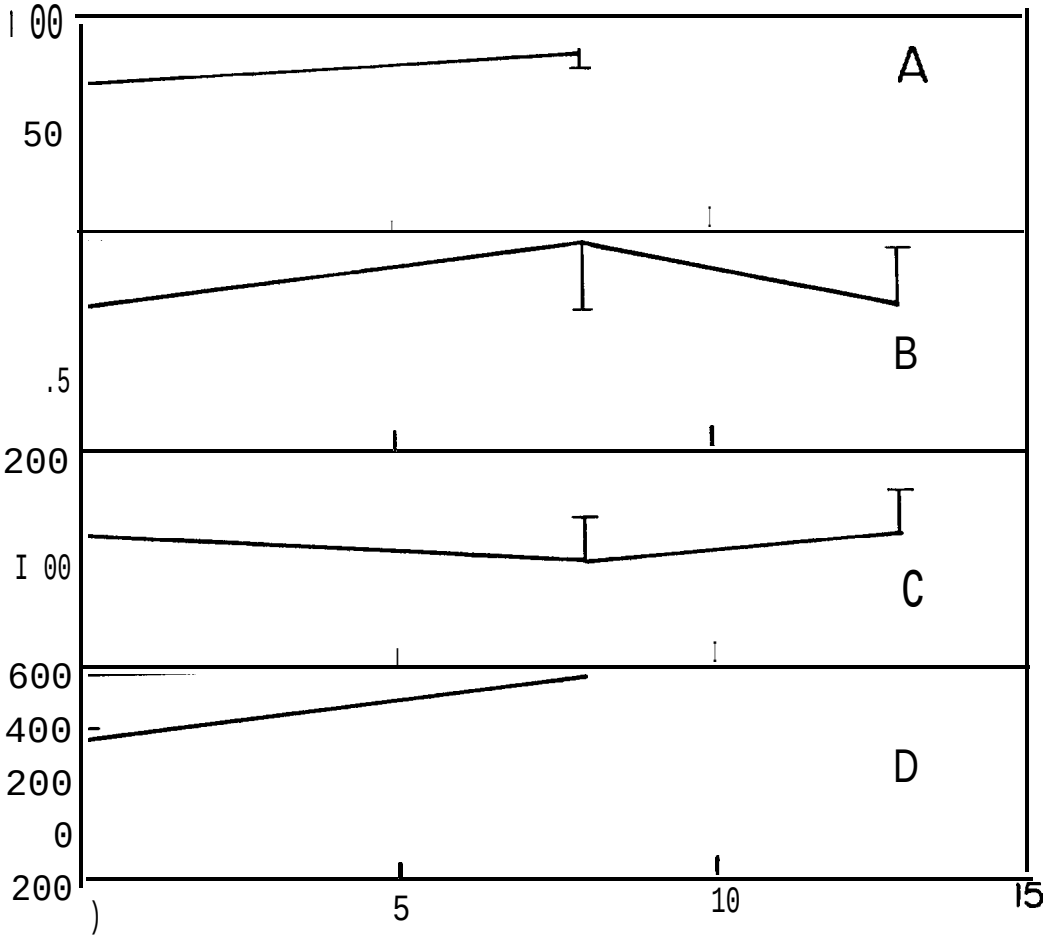

Figure 6A. Values for-assimilation ratio (A); Filtration rate, l/h (B); Respiration rate, ul/h (C) ; and scope for growth (D) for Serripes groenlandicus never exposed to petroleum. Vertical lines are 1 standard deviation.

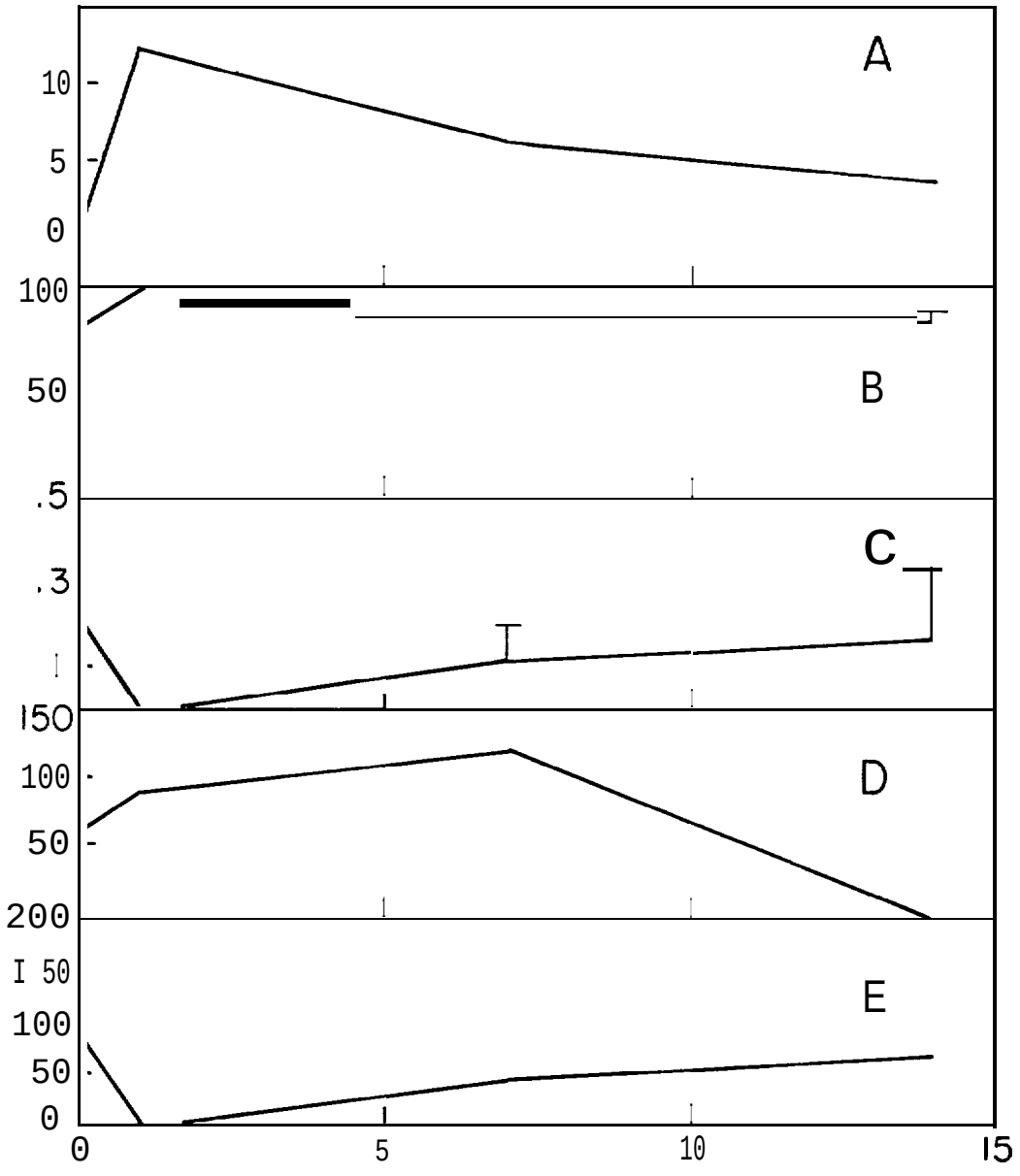

Figure 7A. Concentration of F2 (aromatic) H-C, ppm wet wt. (A); Values for assimilation ratio (B); Filtration rate, l/h (C); Respiration rate, ul/h (D); and scope for growth (E) for Mya truncata exposed to the Bay 9 simulation. Vertical lines are 1 standard deviation.

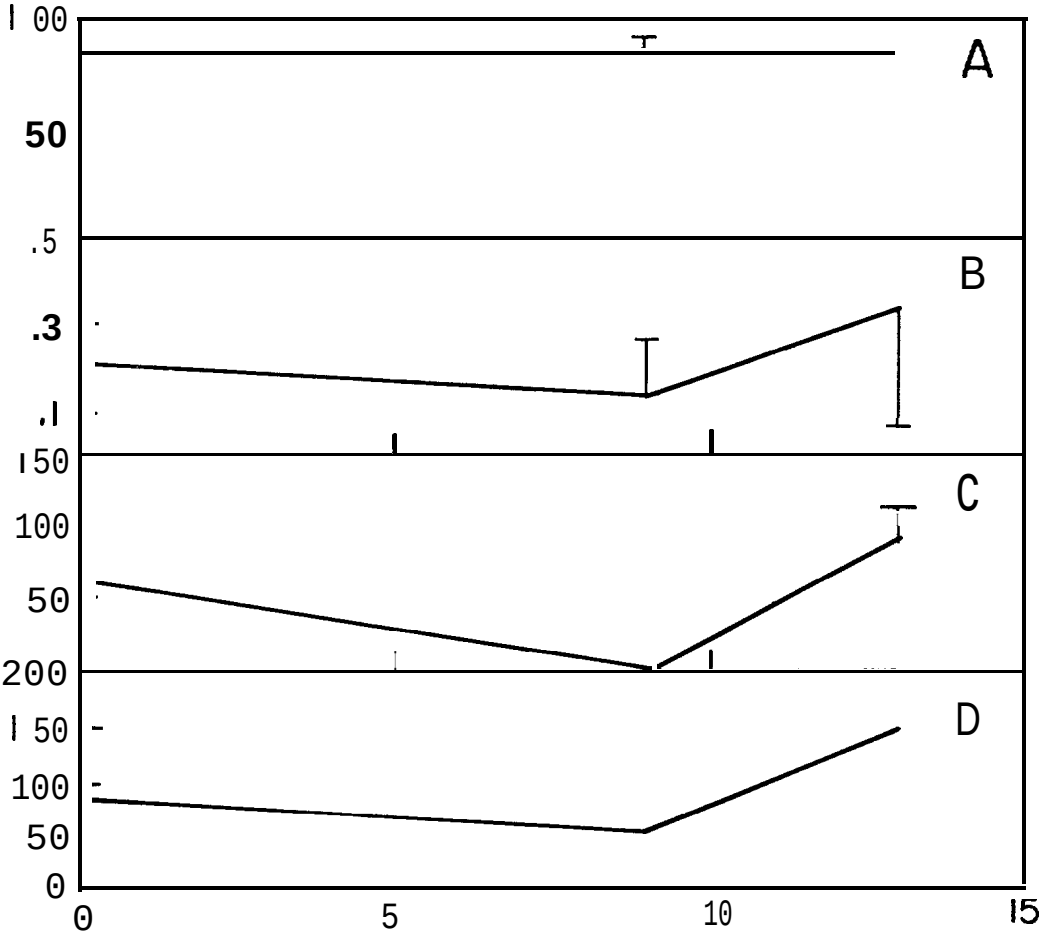

Figure 9A. Values for assimilation ratio (A) ; Filtration rate, l/h (B) ; Respiration rate, ul/h (C); and scope for growth (D) for Mya truncata never exposed to petroleum. Vertical lines are 1 standard deviation.

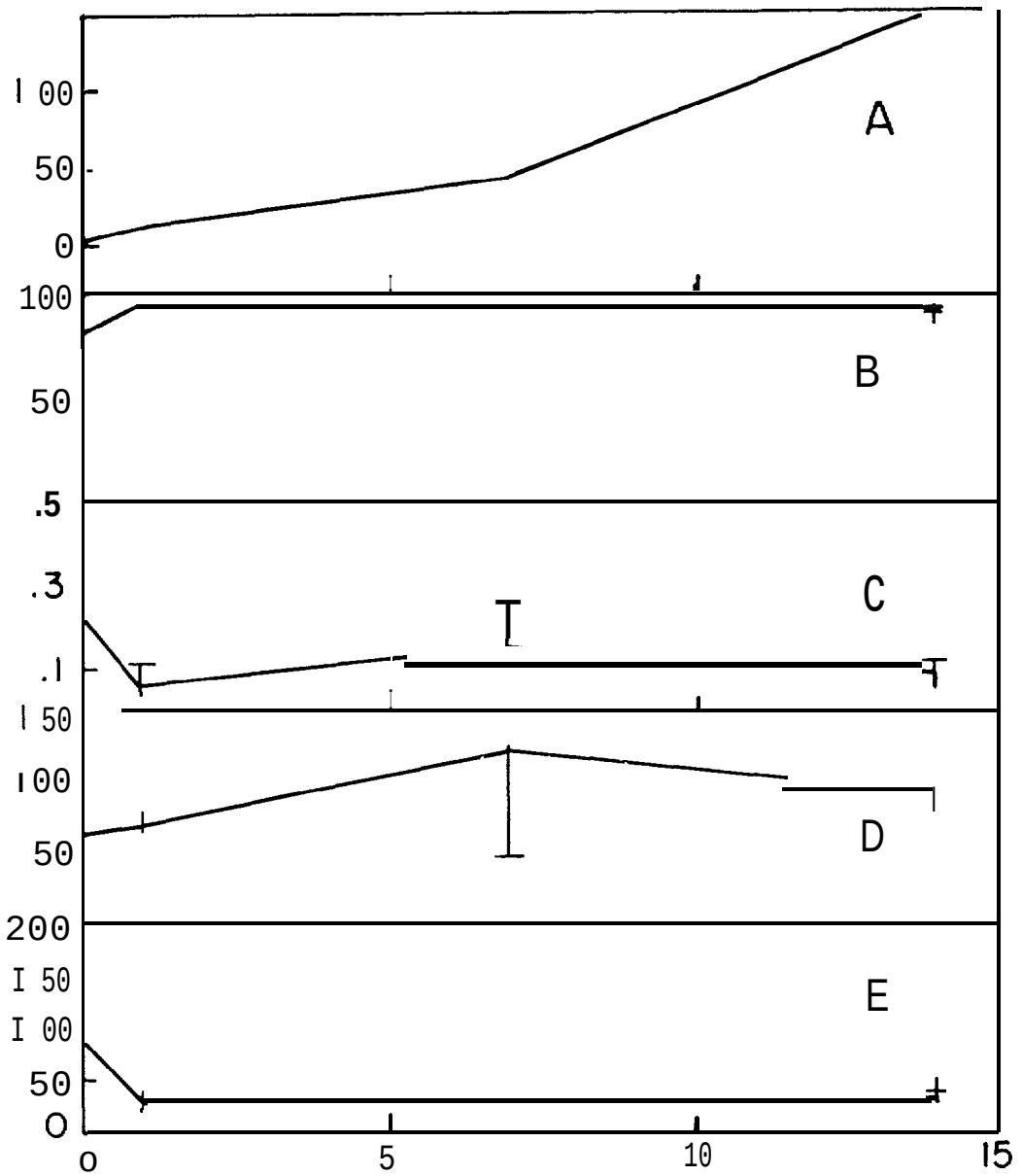

Figure lOA. Concentration of F2 (aromatic) H-C, ppm wet wt. (A); Values for assimilation ratio (B); Filtration rate, l/h (c); Respiration rate, ul/h (D); and net carbon (E) for Mya truncata exposed to 500 ppb chemically dispersed oil. Vertical lines are 1 standard deviation.

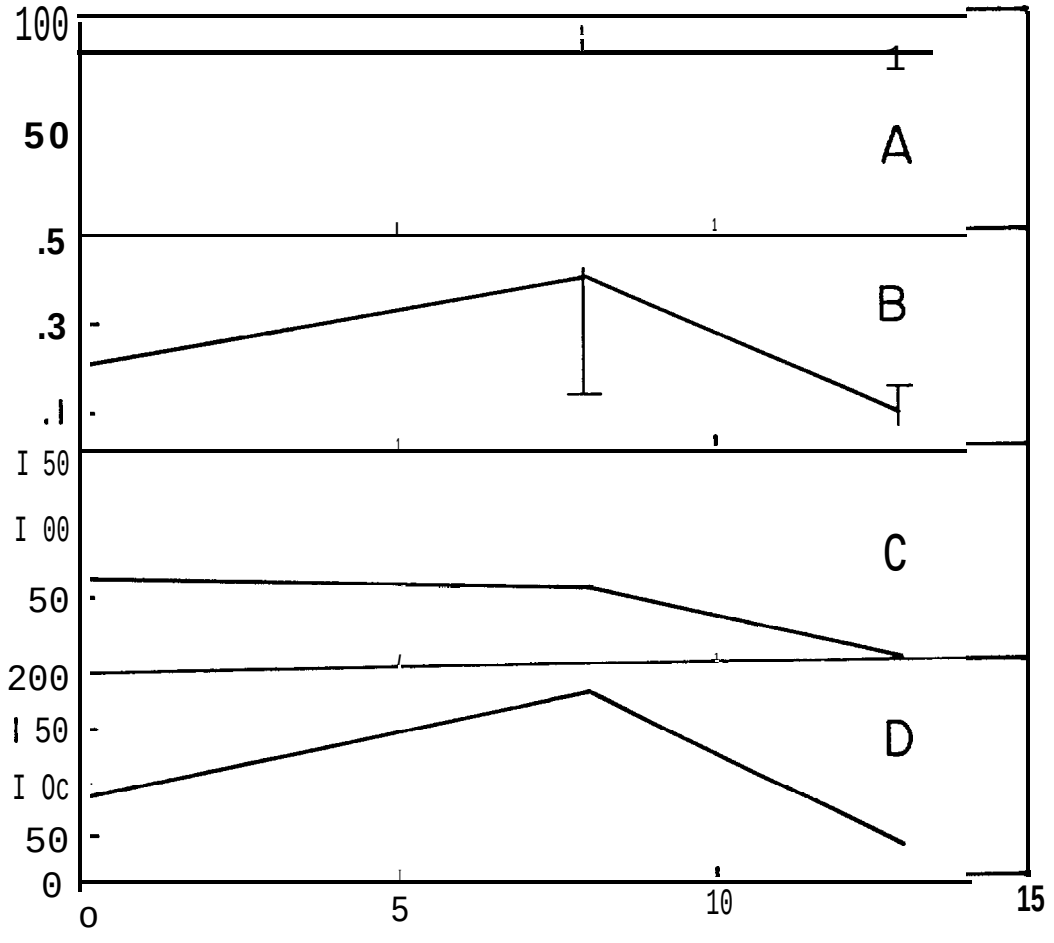

Figure 12A. Values for assimilation ratio (A); Filtration rate, l/h (B) ; Respiration rate, ul/h (C) ; and net carbon (E) for Mya truncata never exposed to petroleum. Vertical lines are 1 standard deviation.

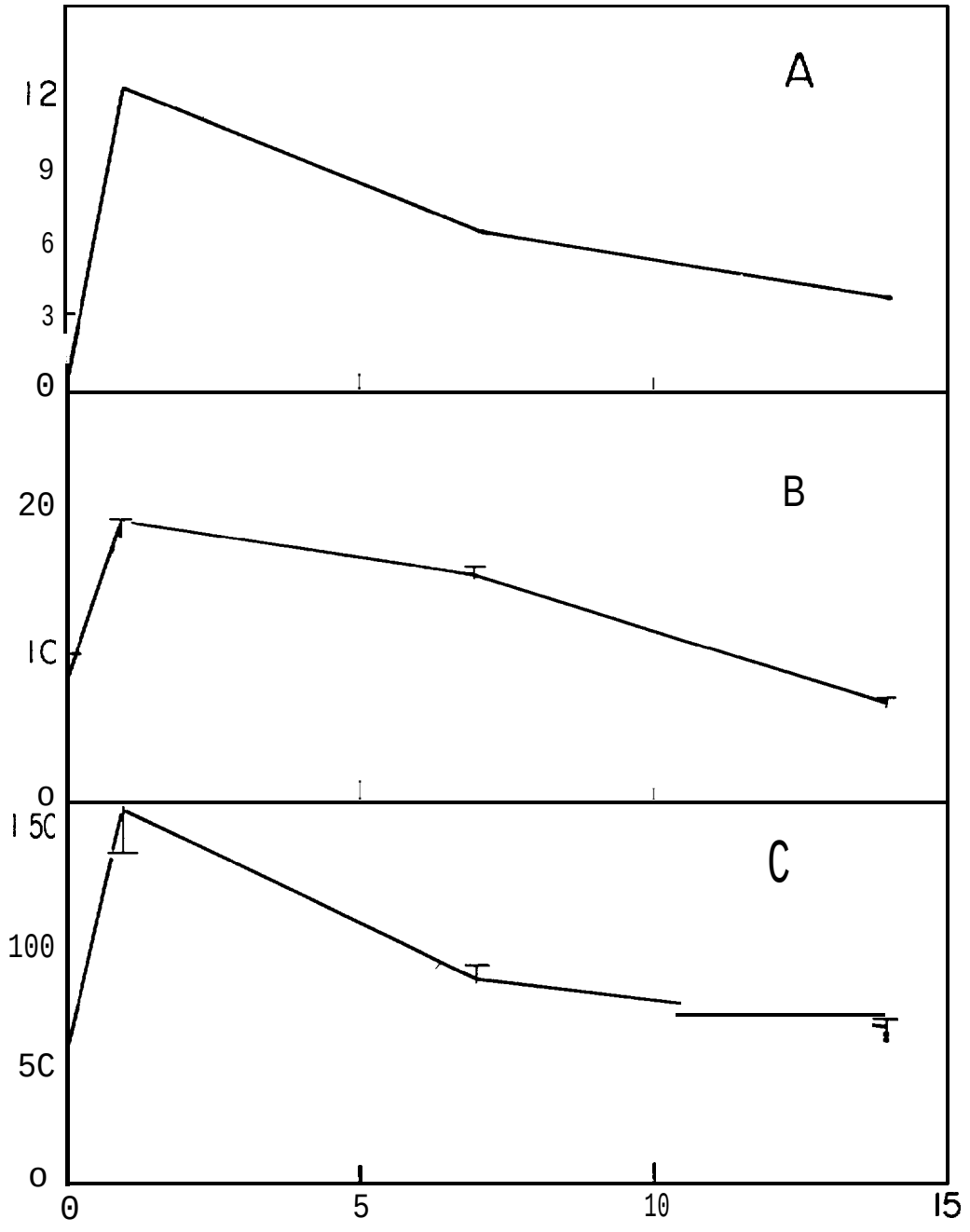

Figure lB. Concentration of F2 (aromatic) H-C, ppm wet wt. (A); activity of G-6-P (B); and AAT (C); for Serripes groenlandicus exposed to Bay 9 simulation. Vertical lines are 1 standard deviation.

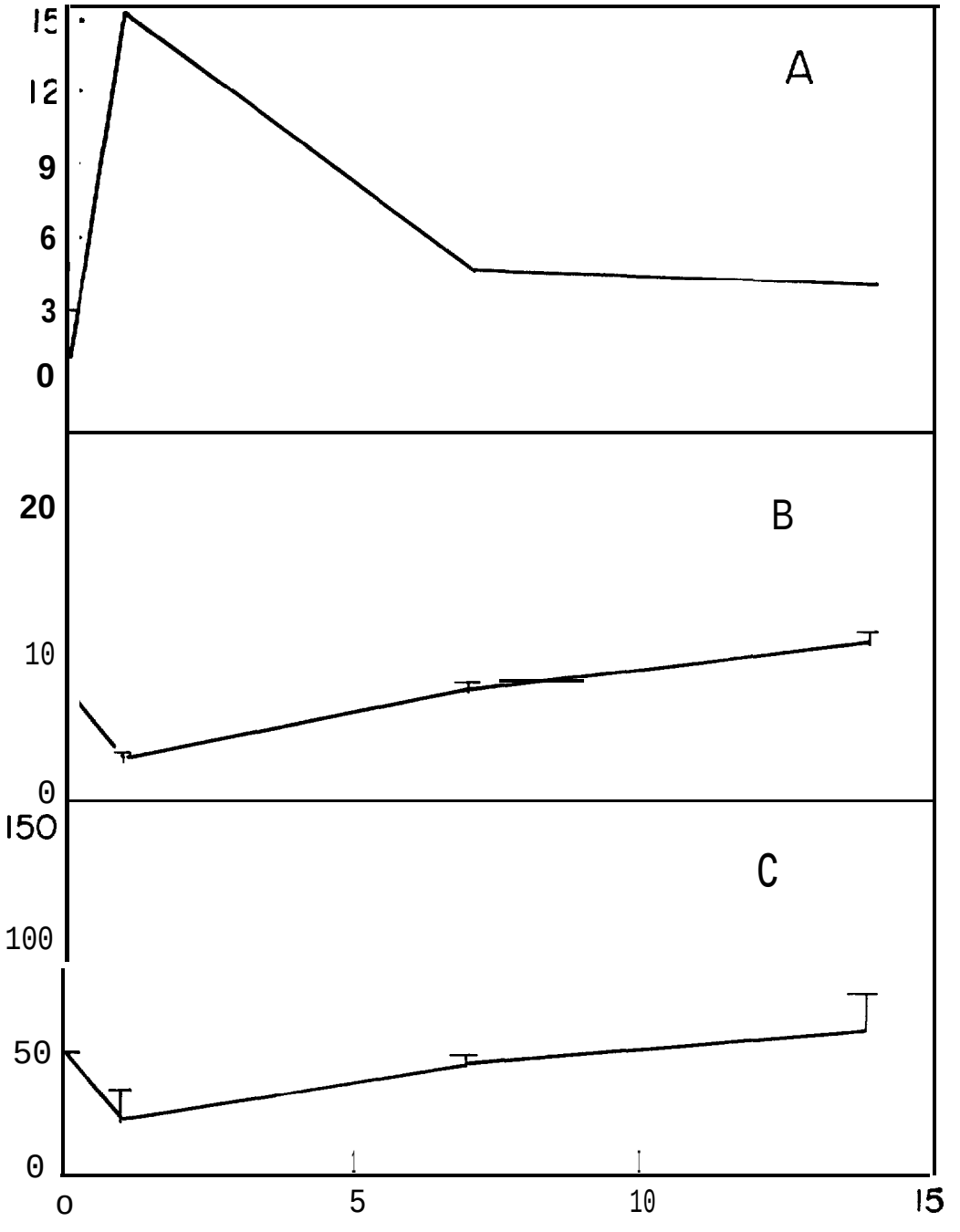

Figure 2B. Concentration of F2 (aromatic) H-C, ppm wet wt. (A); activity of G-6-P (B) and AAT (C) for Serripes groenlandicus exposed to Bay 10 simulation. Vertical lines are 1 standard deviation.

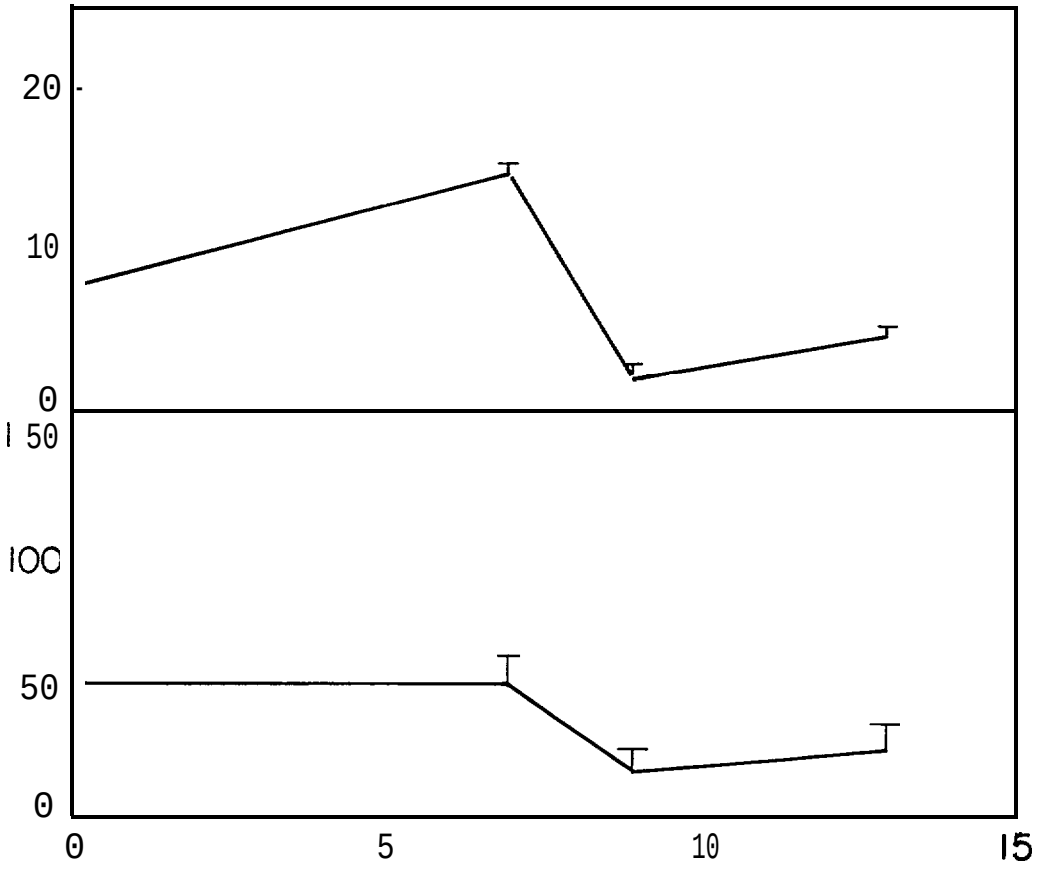

Figure 3B. Activity of G-6-P (A) and AAT (B) for Serripes groenlandicus never exposed to oil. Vertical lines ~~are 1~~ standard deviation.

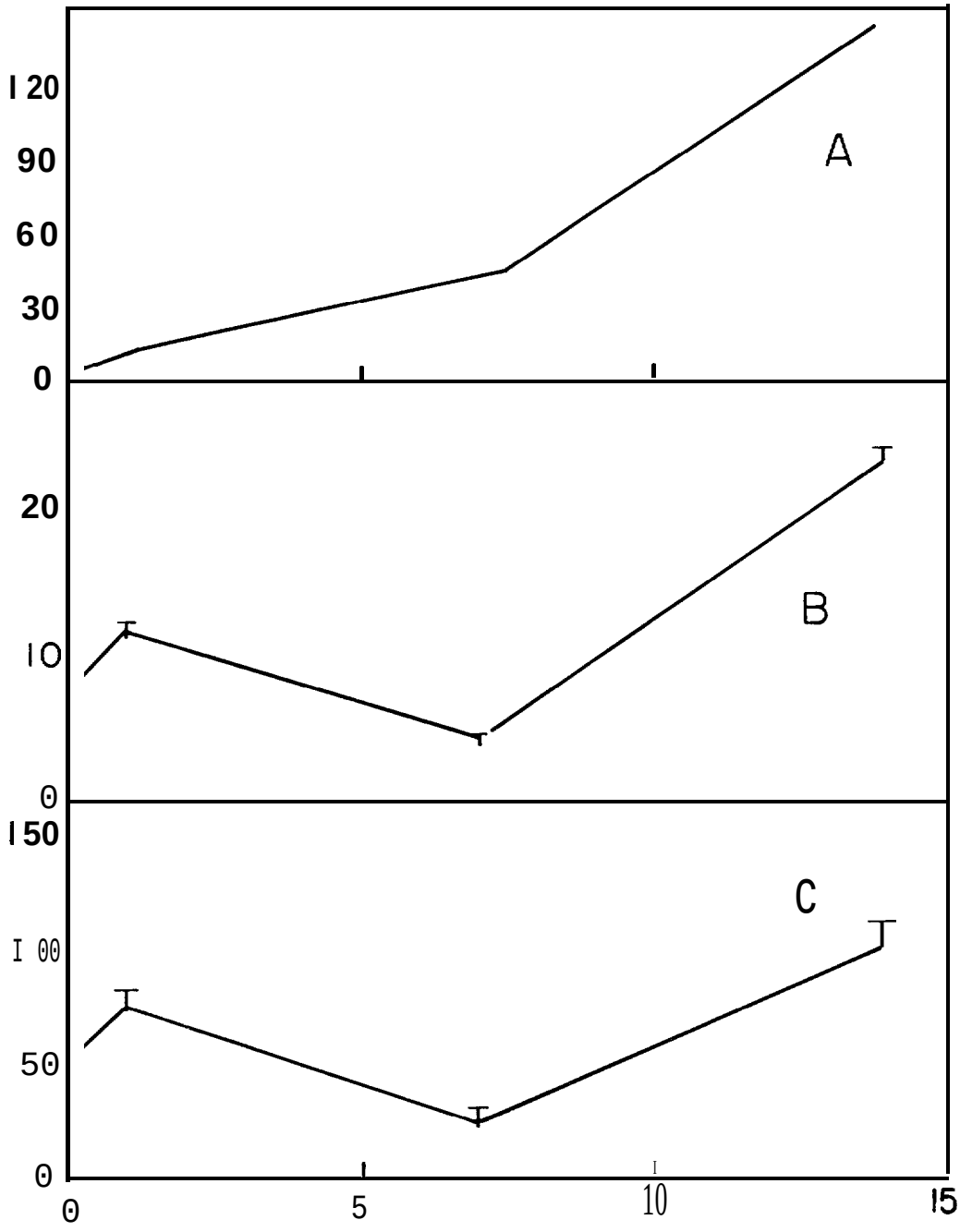

Figure 4B. Concentration of F2 (aromatic) H-C, ppm wet wt. (A); activity of G-6-P (B) and AAT (C) for Serripes groenlandicus exposed to 500 ppb dispersed oil. Vertical lines are 1 standard deviation.

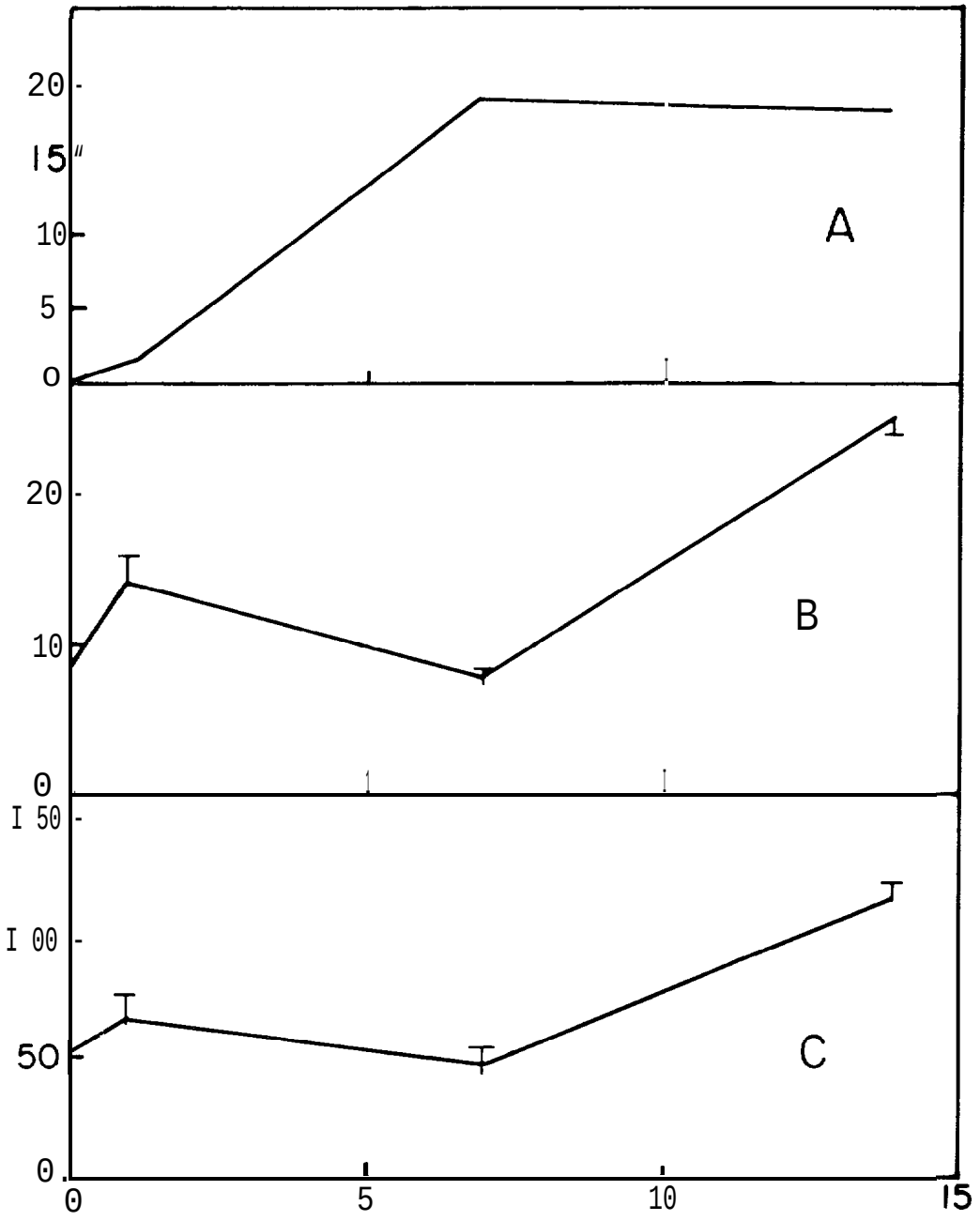

Figure 5B. Concentration of F2 (aromatic) H-C, ppm wet wt. (A); activity of G-6-P (B) and AAT (C) for Serripes groenlandicus exposed to 50 ppb dispersed oil. Vertical lines are 1 standard deviation.

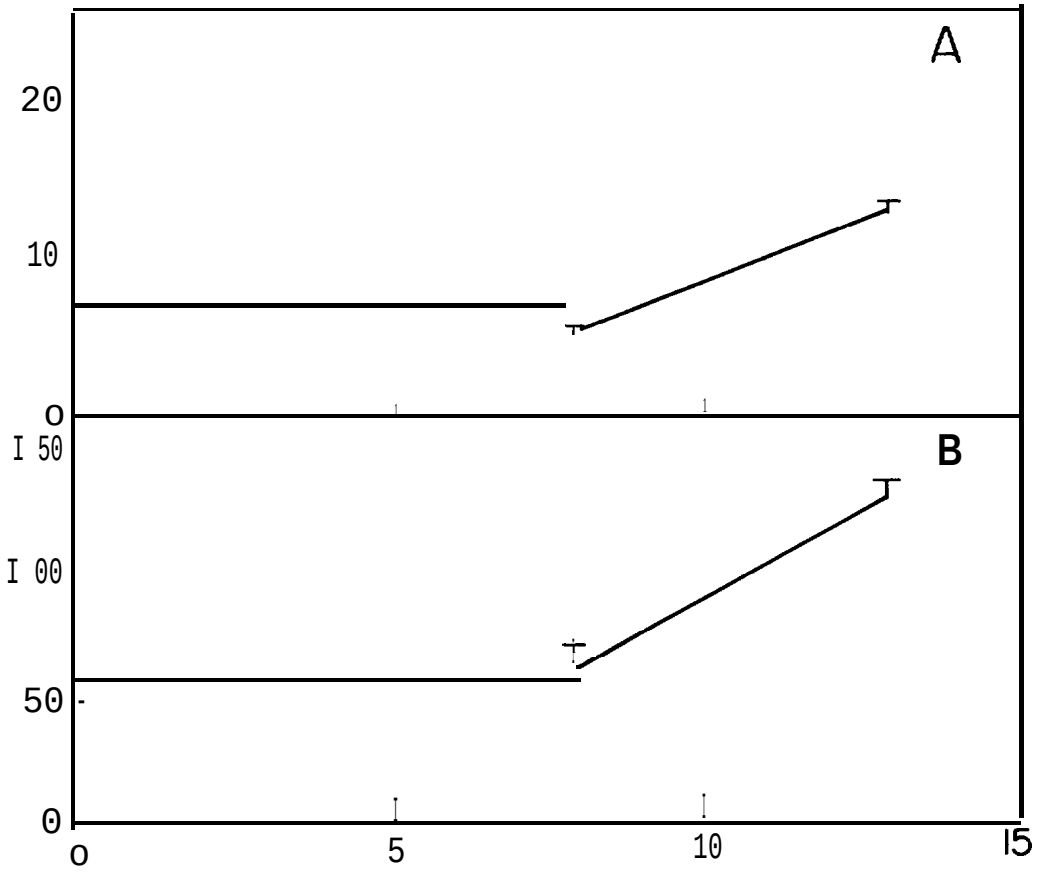

Figure 6B. Activity of G-6-P (A) and AAT (B) for Serripes groenlandicus never exposed to oil. Vertical lines are 1 standard deviation.

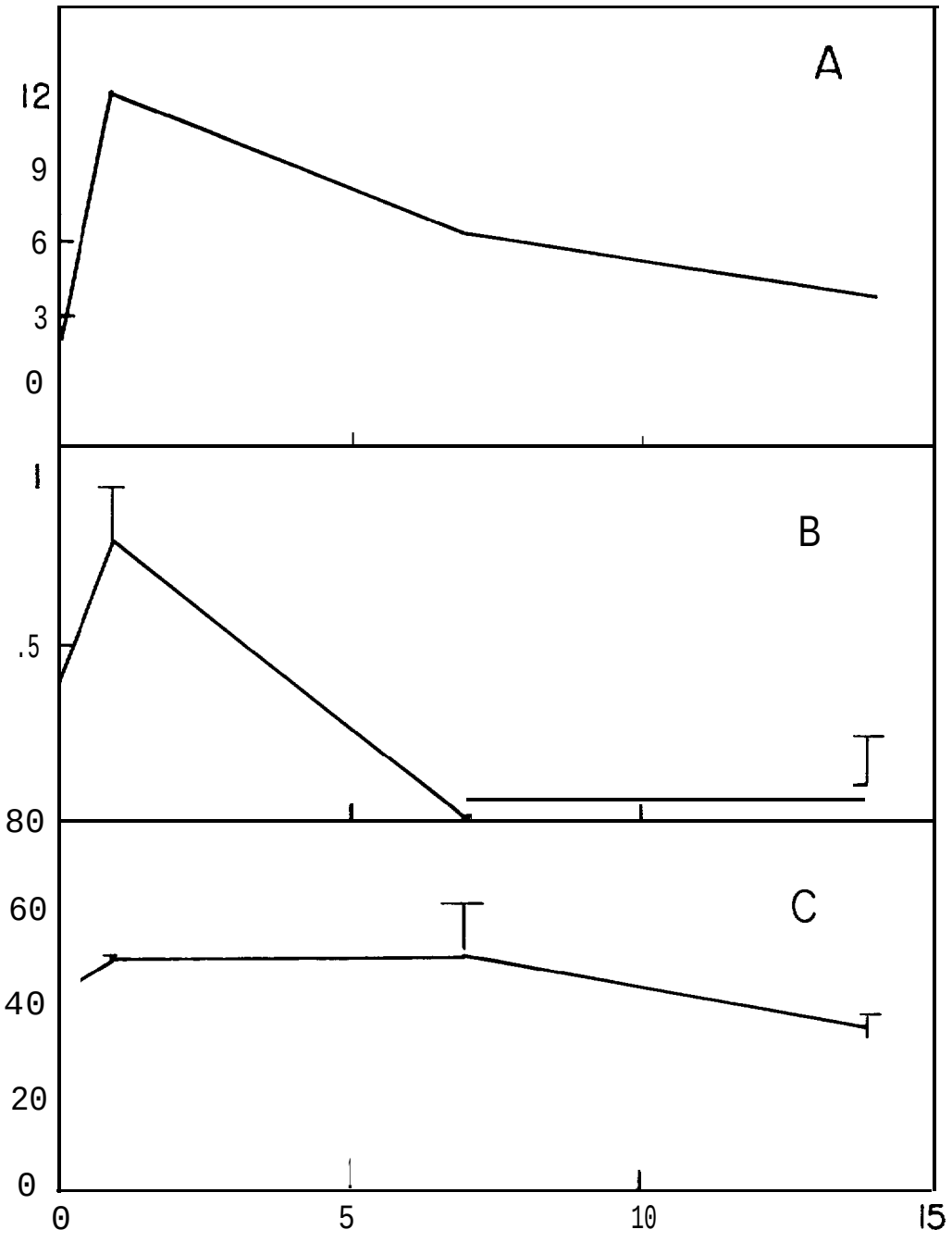

Figure 7B. Concentration of F2 (aromatic) H-C, ppm wet wt. (A); activity of G-6-P (B) and AAT (C) for Mya truncata exposed to Bay 9 simulation. Vertical lines are 1 standard deviation.

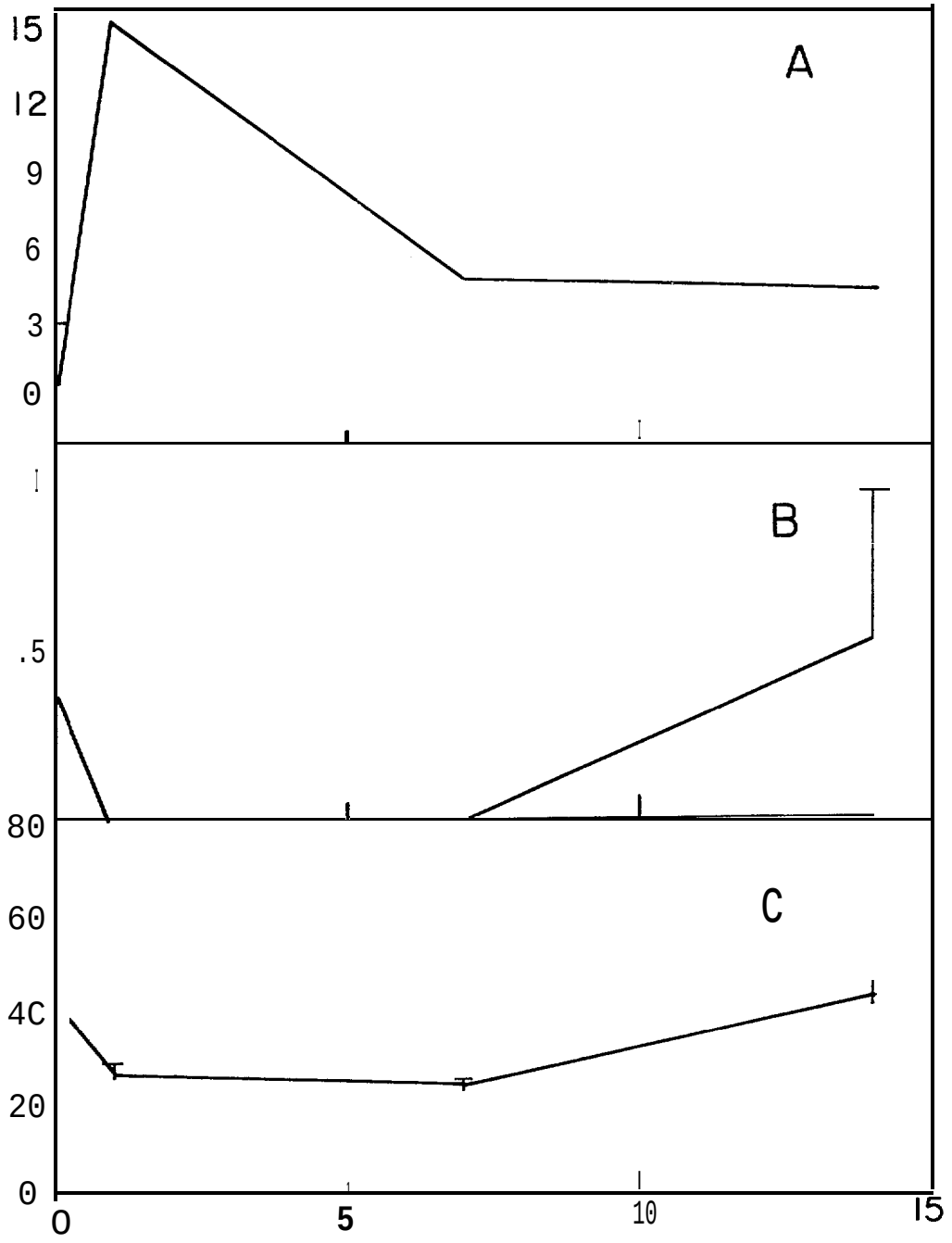

Figure 8. Concentration of F2 (aromatic) H-C, ppm wet wt. (A); activity of G-6-P (B) and AAT (C) for Mya truncata exposed to Bay 10 simulation. Vertical lines are 1 standard deviation.

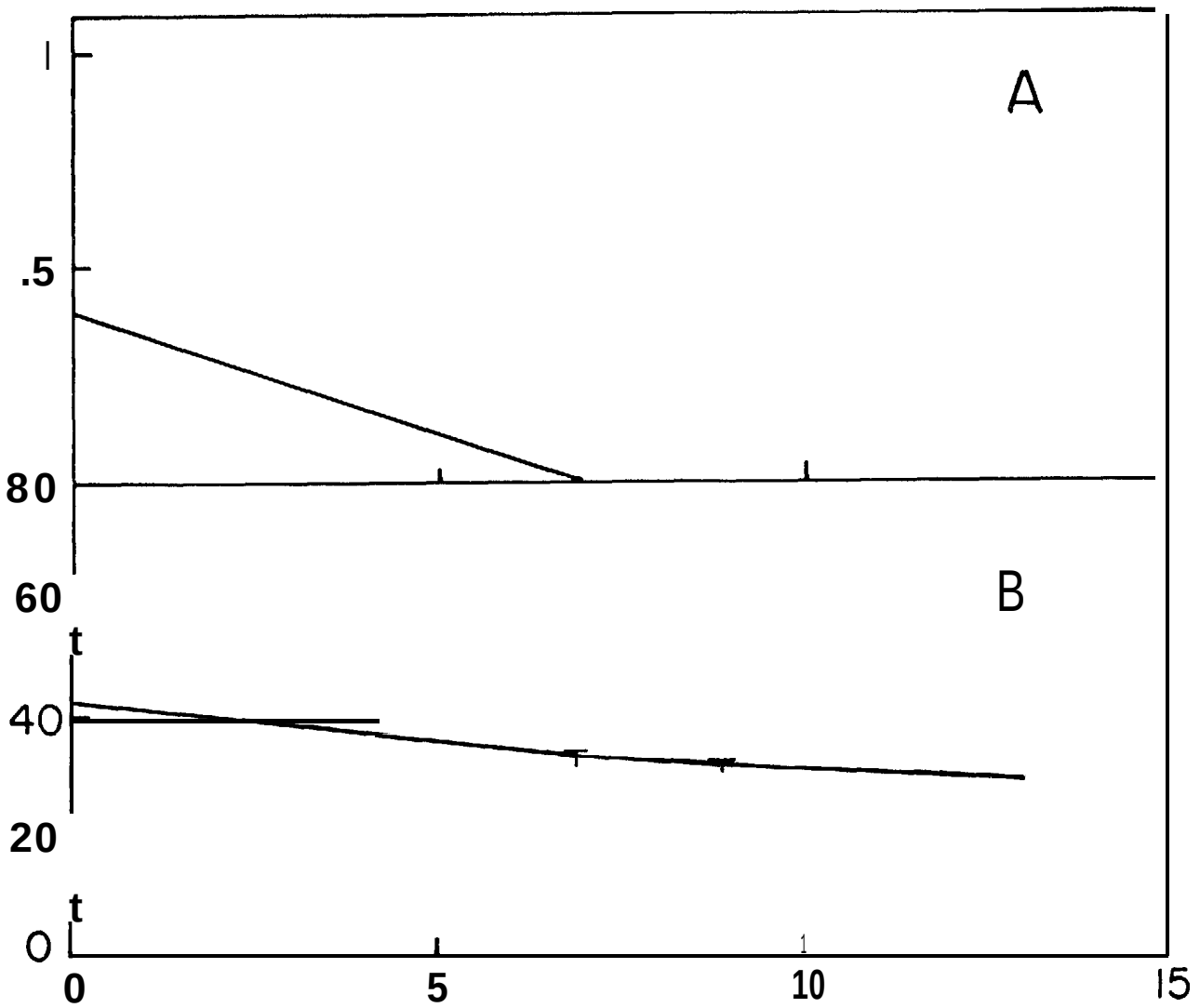

Figure 9B. Activity of G-6-P (A) and AAT (B) for Mya truncata never exposed to oil. Vertical lines are 1 standard deviation.

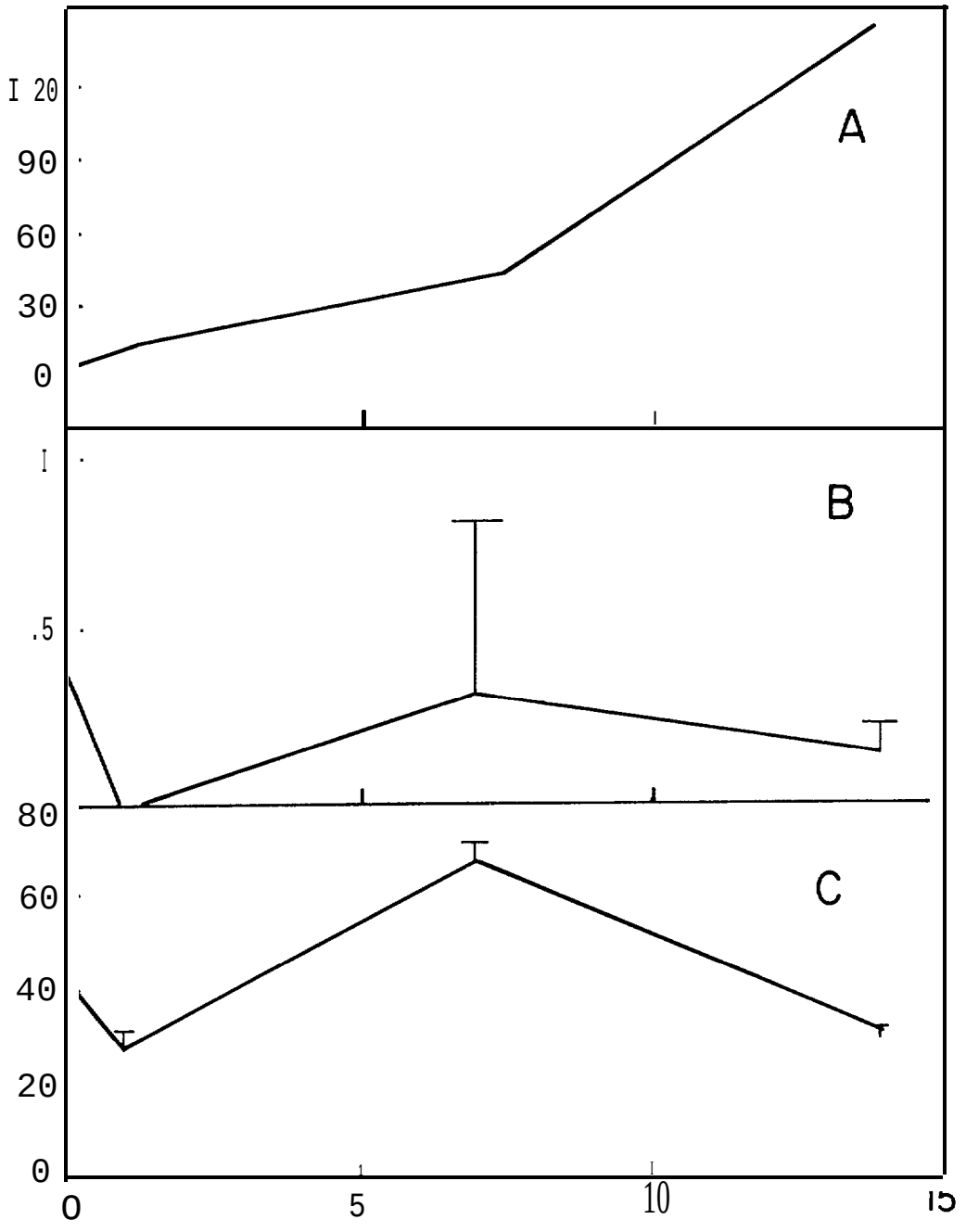

Figure 10B. Concentration of F2 (aromatic) H-C, ppm wet wt. (A); activity of G-6-P (B) and AAT (C) for Mya truncata exposed to 500 ppb dispersed oil. Vertical lines are 1 standard deviation.

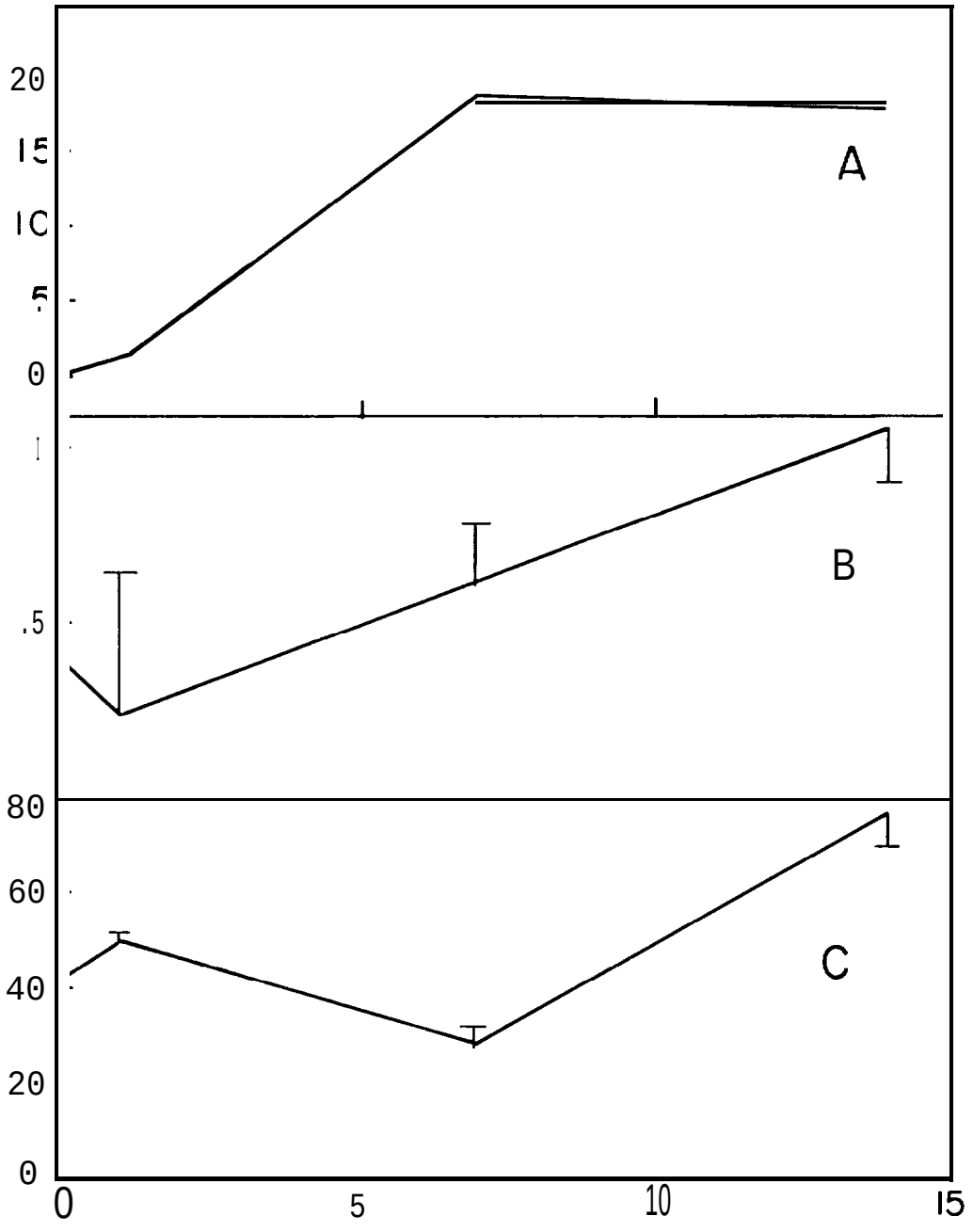

Figure 11. Concentration of F2 (aromatic) H-C, ppm 'wet wt. (A); activity of G-6-P (B) and AAT (C) for Mya truncata exposed to 50 ppb dispersed oil. Vertical lines are 1 standard deviation.

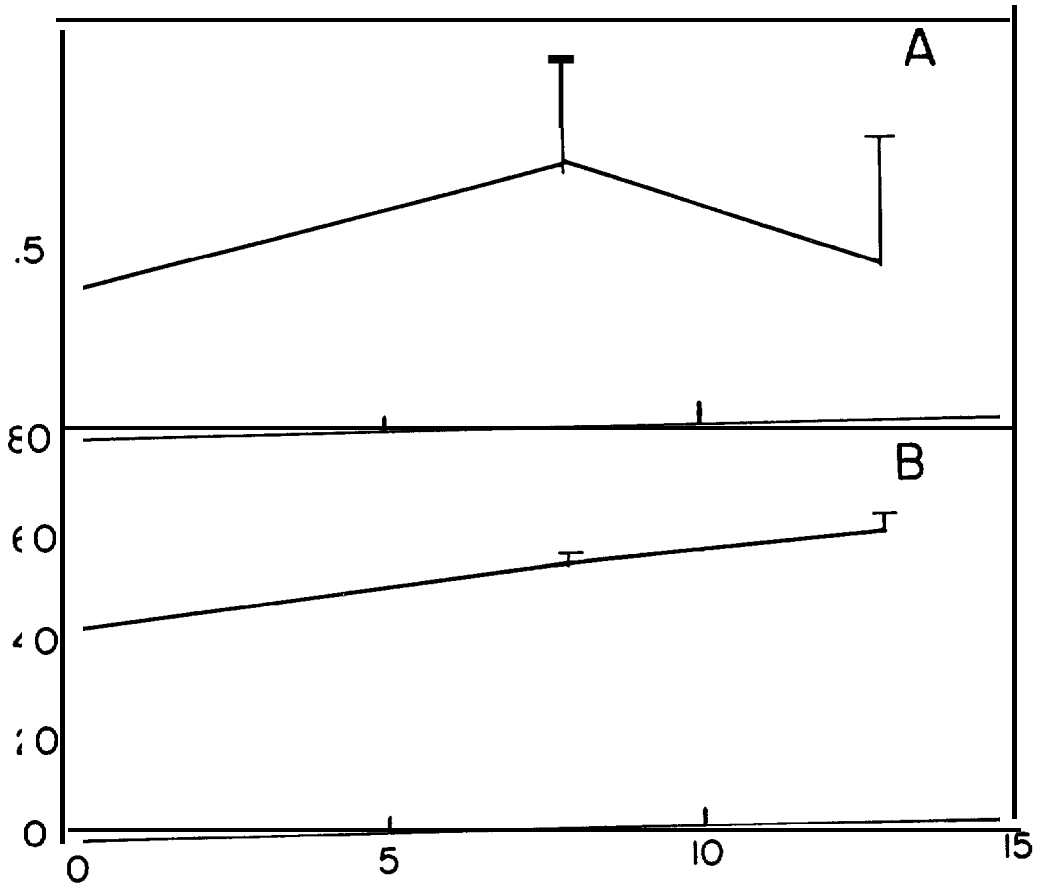

Figure 12B. Activity of G-6-P (A) and AAT (B) for Mya truncata never exposed to oil. Vertical lines are 1 standard deviation.

4.0 DISCUSSION:

The results of this experiment have shown that there exist large differences between Mya truncata and Serripes groenlandicus in their reactions to exposure to dispersed oil. Mya truncata is considerably less sensitive to dispersed oil than is Serripes groenlandicus. Mya truncata is also considerably less tractable as an experimental animal than is Serripes groenlandicus.

There are also large differences in the rate at which Mya truncata and Serripes groenlandicus take up oil from the long term exposures. The data shown in Table 9 suggest that the rate of uptake of dispersed oil from low concentrations is dependent upon the external concentration of oil and upon the animals' filtration rate. It is clear that Serripes groenlandicus filters ca 5 times as much water as does Mya truncata. The ratios of the final (14d) tissue hydrocarbon concentrations approximate that ratio for both 50 ppb and 500 ppb exposures. It is also interesting that the ratio of final body burdens for both species at 500 ppb to final body burdens at 50 ppb approximate 10:1.

With regard to the rate of hydrocarbon loss (deputation) the data shown in Table 9 suggest that the deputation rate is independent of filtration rate. This is to be expected if the deputation rate is a first order phase transfer process.

Both Mya truncata and Serripes groenlandicus may be more

sensitive to dispersed oil than are Mya arenaria and Mytilus edulis. In the results of the Searsport experiment (Gilfillan et al. 1984) no physiological effects caused by dispersed oil were observed in either Mya arenaria or in Mytilus edulis. In Mya truncata and in Serripes groenlandicus significant effects of exposure to dispersed oil were observed. However, the major effects of the dispersed oil exposure were exhibited at the 18 h sampling; no samples were taken at Searsport before 7d .

In those groups of animals subjected to sequential simulations of the Bay 9 and Bay 10 exposures significant reductions were observed in SFG in both Mya truncata (Bay 9 only; NETC) and in Serripes groenlandicus. These changes in SFG could be correlated statistically with the body burden of aromatic hydrocarbons.

Mya truncata exposed to the Bay 10 simulation were not examined. However, when the changes in SFG for Serripes groenlandicus exposed to the Bay 9 simulation (Fig. 1A) are compared with those observed for animals exposed to the Bay 10 simulation (Fig. 2A) it is clear that the reduction in SFG in the Bay 9 exposed animals was double the reduction seen in the Bay 10 exposed animals. Clearly, the greater exposure of the Bay 9 exposed animals has led to a greater reduction in SFG.

In Serripes groenlandicus exposed to both the Bay 9 and the Bay 10 simulations changes were observed in the activity

of AAT and G-6-l?. The changes induced by the greater Bay 9 exposure were larger than those induced by the Bay 10 exposures; they were also opposite in direction. In the Bay 9 exposed animals there was a large increase in the activity of both enzymes. In the Bay 10 exposed animals the activity of both enzymes was reduced. It is not unusual for doses of a toxicant differing by an order of magnitude to have different effects. In both the Bay 9 exposed animals and the Bay 10 exposed animals a significant dose-response relationship was obtained for enzyme activity and the body burden of aromatic hydrocarbons (Table 7) .

In examining the physiological consequences of being confined in the sequential dosing apparatus it is clear that there was little effect on the elements of SFG in Serripes groenlandicus. With regard to enzyme activity the data are noisier, particularly at 14 d. Part of the noise may result from the necessary small tissue samples used; part of the noise may result from the effects of confinement.

The data for AAT and G-6-P in sequentially exposed Mya truncata are very noisy. No dose-response relationships could be shown (Table B). However, the trends observed are the same as those seen in Serripes groenlandicus. In Bay 9 exposed animals' enzyme levels tend to increase; in Bay 10 exposed animals' enzyme levels tend to decrease.

The Bay 9 exposed Serripes groenlandicus did not recover to "control" levels of SFG by day 14; Bay 10 exposed animals did.

All sequentially exposed Serripes groenlandicus recovered to "control" enzyme levels by day 14. Thus the greater exposure had longer lasting effects.

In Mya truncata exposed to the Bay 9 simulation NETC had not recovered to "control" values by day 14. No significant oil induced effects were observed on enzyme levels in sequentially exposed Mya truncata.

In contrast to the relatively clear cut results of the sequential exposures, the results of the long-term exposure experiments are more difficult to explain. No dose response relationships were observed for SFG, AAT activity or G-6-P activity and body burden of aromatic hydrocarbons in either species in any treatment. This result is in spite of the fact that long-term exposed animals had higher body burdens of aromatic hydrocarbons than the sequentially exposed animals. In addition, there is no graded response between 500 and 50 ppb exposure such as was observed between the Bay 9 and Bay 10 exposures in Serripes groenlandicus. The animals do not seem to react the same way to long-term exposure as they do to sequential (acute) exposure.

An explanation for these disparate results may be found by comparing the composition of the petroleum body burden in each of the types of exposure (Boehm, 1984). The physiological effects seen in this study in the sequential exposures are associated with large quantities of alkylated benzene and with

C_1 and C_2 substituted naphthalenes (low boiling aromatics). In Boehm's Figures 9 and 12, large quantities of low boiling aromatics are present in the 18 hr. samples; relatively little low boiling material is present at 21 days. In contrast, in the long-term exposed animals, the relative concentration of low boiling aromatics is never high (Boehm's Figs. 14; 16).

There are two possible explanations for the relative lack of light aromatic hydrocarbons in the tissues of the long-term exposed animals. The first is that, in spite of the precautions taken to prevent loss to the atmosphere, the lighter, more acutely toxic (Neff et al., 1976), aromatic compounds were lost from the long-term exposure solutions. As a result, little effect on AAT, G-6-P or SFT would be expected. The change in composition of the long-term exposure solutions is similar to the compositional changes observed in the dispersed oil in the Searsport experiment (Page et al., 1984). A second explantion could be that because the light aromatic hydrocarbons are more water soluble than heavier compounds and because they were present at very low levels in the water, the lighter aromatics may have partitioned out of the tissues and back into the water. The data at hand are not sufficient to say which possible explanation is correct. It is clear, however, that substantial differences in the relative composition of the body burden of aromatic hydrocarbons exist between sequentially exposed animals and the long-term exposed animals.

The results of this study have shown that:

1. The different filtration rates observed for Mya truncata and Serripes groenlandicus affect the rate at which hydrocarbons are taken up by these species.
2. The differing filtration rates do not appear to affect the deputation rates observed for these species.
3. Both species gave clear dose-response relationships between physiological rates and the body burden of petroleum in the acute (sequential) exposure experiment.
4. No dose-response relationships were observed in the long-term exposure experiments.
5. The lack of dose-response relationships in the long-term exposed animals appears to be related to compositional changes in the body burden of aromatic hydrocarbons as compared to the body burden of the sequentially exposed animals.

BIBLIOGRAPHY

Ansell, A. D. and Lander, K. F. 1967. "Studies on the hard shell clam, Venus mercenaria, in British waters. III. Further observations on the seasonal biochemical cycle and on spawning." J. Appl. Ecol. 4:425-435.

Boehm, P. D. 1984. Analysis of hydrocarbon content and composition of biological tissue and sediment samples from the 1983 DIAND/BIOS tank study. Final report contract #RN A7135-3-0027/ FC1362-1362-0 4727-101-1160-0GD00001/OST83-00194 . Department of Indian Affairs and Northern Development, Ottawa, Ontario, Canada. K1A 0H4, 7 March 1984.

Gilfillan, E.S., D. Mayo, S. Hanson, D. Donovan and L. C. Jiang. 1976. "Reduction in Carbon Flux in Mya arenaria Caused by a Spill of No. 6 Fuel Oil." Mar. Biol. 337:115-123.

Gilfillan, E.S., D. W. Mayo, D.S. page, D. Donovan and S.A. Hanson. 1977. "Effects of varying concentrations of hydrocarbons in sediments on carbon flux in Mya arenaria." in:Physiological Responses of Marine Biota to Pollutants. F. J. Vernberg, A. Calabrese, F.P. Thurberg and W.B. Vernberg eds. Academic Press, New York. xiii + 462 pp.

Gilfillan, E.S., D.S. page, S.A. Hanson, J. Foster, J. Hotham, D. Vallas, and R. Gerber. 1984. Effects of Test Spills of Chemically Dispersed and Non-Dispersed Oil on the Activity of Aspartate Aminotransferase and Glucose-6-Phosphate Dehydrogenase Activity in Two Intertidal Bivalves, Mya

arenaria and Mytilus edulis. In: Oil Spill Dispersants: Five Years of Research, ASTM, Philadelphia, PA.

Gould, E. 1977. "Alteration of enzymes in winter flounder, Pseudopleuronectes americanus, exposed to sublethal amounts of cadmium chloride." in:Physiological Responses on Marine Biota to Pollutants. F. J. Vernberg, A. Calabrese, F. P. Thurberg and W. B. Vernberg eds. Academic Press, New York. xiii + 462 pp.

Hemmingson, A.M. 1950. "The relationship of standard (basal) energy metabolism to total fresh weight in living organisms." Rep. Steno. Hosp. Copenhagen. 4:7-58.

Neff, J.M., B.A. Cox, D. Dixit, and J. W. Anderson. 1976. Accumulation and release of petroleum derived hydrocarbons by four species of marine animals. Marine Biology, 38, 279-289.

Page, D.S., J.C. Foster, J.R. Hotham, D. Vallas, E.S. Gilfillan, S.A. Hanson, and R.P. Gerber. 1984. Tidal Area Dispersant Project: Fate of Dispersed and Undispersed Oil in Two Near-shore Test Spills. In:Oil Spill Dispersants: Five Years of Research, ASTM, Philadelphia, PA.

Thurberg, F.P., A. Calabrese, E. Gould, R.A. Greig, M.A. Dawson and R.K. Tucker. 1977. in:Physiological Responses of Marine Biota to Pollutants. F.J. Vernberg, A. Calabrese, F.P. Thurberg and W.B. Vernberg eds. Academic Press, New York. xiii + 462 pp.

Zeuthen, F. 1947. “Body size and metabolic rate in the animal kingdom with special regard to the marine microfauna." Comptes Rendues des Travaux du Laboratoire, Carlsberg. Seri Chimique 26:17-161.

Zeuthen, F. 1953. “Oxygen uptake as related to body size in organisms .“ Quart. Rev. Biol. 28: 1-12.